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*Study Title***ENFORCEMENT METHOD FOR THE DETERMINATION OF METHOMYL IN DRY PEA SEED AND HAY, SORGHUM FORAGE AND HAY, SOYBEAN HAY, AND SUGAR BEET FOLIAGE***Data Requirement*

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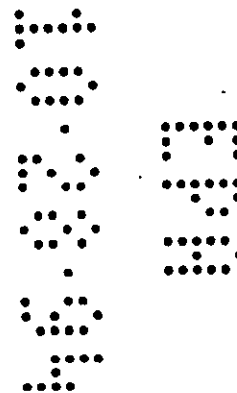
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Statement of No Data Confidentiality Claims

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B), or (C).

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GOOD LABORATORY PRACTICE STATEMENT

The EPA Good Laboratory Practice (GLP) requirements specified in 40 CFR Part 160 are not applicable to analytical methods development.

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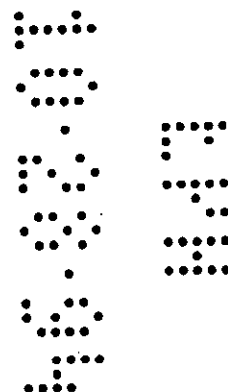


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ENFORCEMENT METHOD FOR THE DETERMINATION OF METHOMYL IN DRY PEA SEED AND HAY, SORGHUM FORAGE AND HAY, SOYBEAN HAY, AND SUGAR BEET FOLIAGE*Janet C. Rühl and Susan Clark***ABSTRACT**

An analytical method using an HPLC with post-column derivatization and fluorescence detection was developed for the determination of methomyl in dry pea seed and hay, sorghum forage and hay, soybean hay, and sugar beet foliage. The limit of quantitation of the method was 0.020 ppm in each of the six matrices.

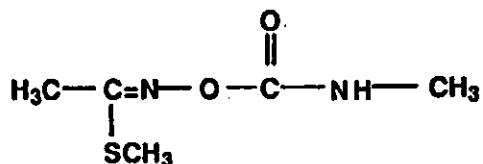
The range of recoveries found for each of six matrices are summarized below with the mean recoveries.

<u>Crop Matrix</u>	<u>Number of Fortifications</u>	<u>Range of Recoveries</u>	<u>Mean % Recovery ± Standard Deviation</u>
pea seed	9	75-90%	83% ± 5%
pea hay	9	82-100%	88% ± 5%
sorghum forage	9	75-105%	90% ± 9%
sorghum hay	9	76-100%	90% ± 8%
soybean hay	16	85-145%*	98% ± 14%
sugar beet foliage	21	79-100%	87% ± 7%

* Recoveries were not corrected for methomyl detected in the control samples. Two high recoveries (135% and 145%) were reported because some of the controls from the magnitude of residue study contained methomyl.

INTRODUCTION

Methomyl is a carbamate insecticide effective against a broad range of agricultural insect pests. This insecticide is registered on a wide range of field crops, vegetables, fruits, and ornamental plants. Its structure appears below.

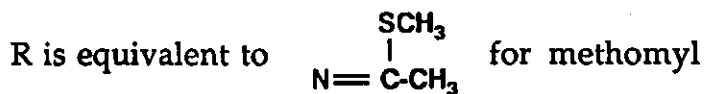


methomyl

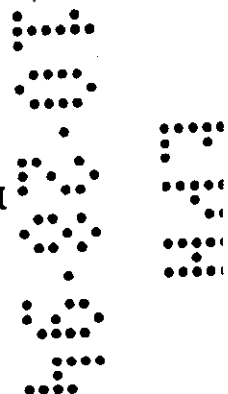
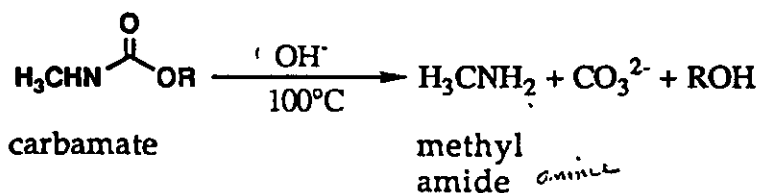
S-methyl N-[(methylcarbamoyl)oxy]thioacetimidate
(CAS Registry No.: 16752-77-5. Also known as DPX-X1179.)

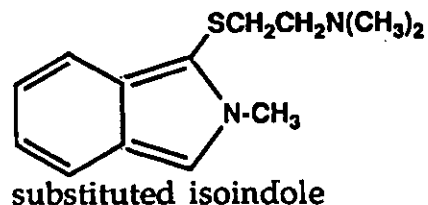
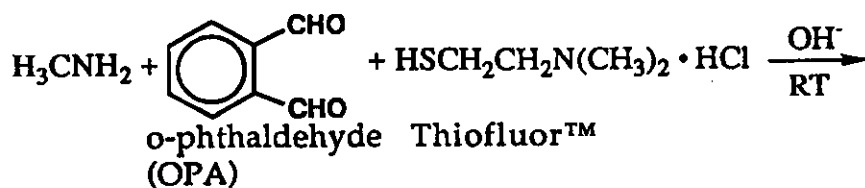
This method, recommended for monitoring methomyl in dry pea seed and hay, sorghum forage and hay, soybean hay, and sugar beet foliage, is based on an earlier method for determining methomyl in grapes (Reference 1). For all matrices, the method involves an acetonitrile extraction, an acetonitrile/hexane partition, and a cleanup on Florisil™ before the extract is analyzed by HPLC with post-column derivatization with fluorescence detection.

The post-column reactions are illustrated as follows where



Hydrolysis:



Derivatization:

The substituted isoindole is highly fluorescent and is detected as a measure of methomyl present.

MATERIALS

During analyses, equivalent equipment, solvents, and glassware may be substituted for those specified in the method. When substitutions are made, equivalent method performance must be verified.

Equipment:

1. Balances:

- | | |
|----------------------|--|
| Analytical balance: | Capable of weighing 0.0001 g for weighing analytical standards. |
| Top-loading balance: | Capable of weighing 0.01 g for weighing samples and sodium chloride. |

2. Homogenizer:

PowerPulse High Speed Homogenizer (N-Phase, Austin, Texas)

3. Evaporators:

Steambath with vacuum

N-EVAP™ Solvent Reduction System (Organomation Associates Inc., Berlin, Massachusetts)

4. Vortex mixer (VWR Scientific, Bridgeport, New Jersey)

5. Solid-Phase Extraction:

Vac Elut SPS 24™ Vacuum Manifold (Varian Associates, Inc., Harbor City, California)

B & J Inert SPE system PTFE flow control valves (Baxter Healthcare Corp., Burdick & Jackson Division, Muskegon, Michigan)

500 mg Florisil® Bond Elut LRC® solid phase extraction cartridge (Varian Associates, Inc., Harbor City, California)

6. Liquid Chromatograph:

Linear LC-304 Fluorescence Detector with SP8800 Ternary HPLC pump, equipped with Pickering PCX 5000 Post Column Reaction Module

Integrator: Spectra-Physics ChromJet SP4400

Auto-sampler: Spectra-Physics SP8880

-OR-

FL 2000 Detector with P4000 Quaternary Gradient pump, equipped with Pickering PCX5100 Post Column Reaction Module

Integrator: Spectra Physics ChromJet SP4400

Auto-sampler: AS 3000 Thermo Separation Products

The above instrument systems are equivalent.

7. HPLC Column:

25-cm X 4.6-mm i.d. x 0.25" o.d. DuPont Zorbax® Rx C8 (5-µm particle size)

8. Autosampler vials:

1.8 mL, with open top screw caps containing Teflon®-coated silicone septa (Thermo Separation Products, Fremont, California)

9. Syringes:

For fortifying samples, preparing standards, performing HPLC injections:

assorted microliter sizes (Hamilton Co., Reno, Nevada)

For filtering samples prior to HPLC injection:

Gastight 1002RN microliter syringe with Teflon®
luer lock: 2.5 mL (Hamilton Co., Reno, Nevada)

10. Filtration:

Samples after acetonitrile extraction

Buchner funnel: 9 cm

Filtering flask: 500 mL

Whatman #4, 9 cm filter paper (VWR Scientific,
Bridgeport, New Jersey)

Air Cadet® vacuum pump/compressor (Cole-
Parmer® Instrument Company, Niles, Illinois)

Samples for HPLC

Millipore 0.45 µm HV Durapore (PVDF) filters
(Millipore Corp., So. San Francisco, California)

Hydrolysis Reagent, 0.2 N NaOH

Filtering flask: 1000 mL

Vacuum filter holder: 300 mL (Whatman LabSales,
Hillsboro, Oregon)

0.45 µm nylon 66 membrane filters, 47 mm (Alltech
Associates, Deerfield, Illinois)

Air Cadet® vacuum pump/compressor (Cole-
Parmer® Instrument Company, Niles, Illinois)

11. Glassware:

Volumetric flasks, class A: assorted sizes

Erlenmeyer flask with stopper: 250 mL

Glass separatory funnel with Teflon® stopcocks: 125 mL

Beakers: 150 mL

Clear Boston Round bottles with Teflon®-lined screw
caps: 100 mL

13 x 100 mm glass culture tubes with Teflon®-lined
screw cap lids

Assorted laboratory glassware

8 oz Qorpak jars with Teflon®-lined lid (Fisher
Scientific, Fair Lawn, New Jersey)

12. Pipettes:

Volumetric, class A pipettes: 20 mL, 5 mL, 1 mL

Graduated pipettes: assorted sizes

Disposable pasteur pipettes

Reagents and Standards

1. Deionized Water (Morse Laboratories, Inc., Polymetric DI Water System)
2. Acetonitrile; (ACN) Fisher HPLC Grade (Fisher Scientific, Fair Lawn, New Jersey)
3. Sodium Chloride Crystals; BAKER ANALYZED® Reagent (J. T. Baker Chemical Co., Phillipsburg, New Jersey)
4. Hexane; OmniSolv® glass distilled (EM Science, Cherry Hill, New Jersey)
5. Dichloromethane; OmniSolv® glass distilled (EM Science, Cherry Hill, New Jersey)
6. Acetone; OmniSolv® glass distilled (EM Science, Cherry Hill, New Jersey)
7. 1-Decanol; BAKER ANALYZED® (J. T. Baker Chemical Co., Phillipsburg, New Jersey)
8. Water; B & J Brand High Purity Solvent (Baxter Healthcare Corp., Burdick & Jackson Division, Muskegon, Michigan)
9. Sodium Hydroxide; Pellets, BAKER ANALYZED® Reagent (J. T. Baker Chemical Co., Phillipsburg, New Jersey)
10. O-Phthalaldehyde; chromatographic grade (Pickering Laboratories, Inc., Mountain View, California)
11. Methanol; B & J Brand High Purity Solvent (Baxter Healthcare Corp., Burdick & Jackson Division, Muskegon, Michigan)
12. N,N-Dimethyl-2-mercaptoethylamine-hydrochloride (Thiofluor); Chromatographic grade (Pickering Laboratories, Inc., Mountain View, California)
13. O-Phthalaldehyde Diluent (0.05 M sodium borate, pH 9.1); (Pickering Laboratories, Inc., Mountain View, California)

14. Methomyl:

analytical grade (E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware; DPX-X1179-379)

For extraction efficiency only: radiolabelled (NEN Lot No. 2995-105; DuPont HOTC No. 426)

15. Helium or other inert gas

16. Nitrogen

Preparation of Solutions

1. Acetone:Hexane (10:90) v/v
2. Acetone:Hexane (50:50) v/v
3. 1% 1-Decanol in acetone: Add 0.25-mL 1-decanol to a 25-mL volumetric flask. Dilute to 25 mL with acetone.
4. Acetonitrile:HPLC grade water (15:85) v/v
5. Hydrolysis reagent, 0.2 N NaOH: Add 8-g NaOH pellets to a 1-liter volumetric flask. Dilute to 1 liter with HPLC grade water. Prior to use, filter the solution using a 1000-mL filter flask, vacuum filter holder and 0.45- μ m nylon 66-membrane filter to remove any particulates and to degas the solvent.
6. Derivatization Reagent: Degas the o-phthalaldehyde diluent (~1 quart) with helium for at least 10 minutes. Weigh approximately 500-mg o-phthalaldehyde into a small beaker and add approximately 10-mL methanol. Stir with a glass rod to break apart lumps and facilitate dissolution. Weigh approximately 2.5-g N,N-dimethyl-2-mercaptoethylamine. Add the o-phthalaldehyde and the N,N-dimethyl-2-mercaptoethylamine to the degassed diluent, rinse the beaker with 1 to 2 mL additional methanol and add to the diluent. Swirl the solution and use when homogeneous.

Note: The thiofluor salt mentioned above is a replacement for liquid 2-mercaptoethanol, and helps eliminate the stench associated with the liquid. However, if the thiofluor is not available, 2-mercaptoethanol may be used: to 500 mg o-phthalaldehyde, add 10-mL methanol and 1 to 2-mL 2-mercaptoethanol. Mix until homogeneous and add to the degassed diluent.

7. Standard Preparation:

Stock Standard Solution

Dissolve nominally 0.1 g methomyl standard in 100 mL of acetonitrile to give a 1000 $\mu\text{g/mL}$ solution. The amount of methomyl standard used should be calculated to correct for less than 100% purity of the standard. For example, if the standard purity is 98.6%, weigh 0.101 g methomyl standard to make the 1000 $\mu\text{g/mL}$ solution.

Fortification Standard Solution

Serially dilute the stock standard solution with acetonitrile to make 100 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$ and 1.0 $\mu\text{g/mL}$ solutions. Other concentrations are similarly prepared as appropriate.

Chromatographic Standard Solutions

Prepare in acetonitrile:HPLC grade water (15:85) and store in amber glass bottles with Teflon[®]-lined screw caps. Suggested concentrations are: 0.1 $\mu\text{g/mL}$, 0.05 $\mu\text{g/mL}$, 0.02 $\mu\text{g/mL}$ and 0.007 $\mu\text{g/mL}$. Suggested preparation is as follows:

Chromatographic Standard Concentration ($\mu\text{g/mL}$)	Fortification Standard Solution Volume (μL)	Fortification Standard Solution Concentration ($\mu\text{g/mL}$)	Volume of Dilution (mL)
0.1	250	10	25
0.05	125	10	25
0.02	50	10	25
0.007	175	1.0	25

ANALYTICAL PROCEDURES

Method

A. Extraction

1. Weigh 10.0 g preprocessed (homogenized) samples into Qorpak jars. Fortify appropriate samples at this time. Suggested fortification standard solution

volumes and concentrations are given below for various fortification levels.

Fortification Level (ppm)	Fortification Standard Solution Volume (mL)	Fortification Standard Solution Concentration ($\mu\text{g/mL}$)	Sample Weight (g)
0.20	0.20	10	10
0.40	0.40	10	10
1.0	1.0	10	10
2.0	0.20	100	10
10	1.0	100	10
20	2.0	100	10

2. Add 10 mL of deionized water and allow sample to soak, without stirring, for 10 minutes.
3. Add 100 mL of acetonitrile and blend at high speed for 2 minutes using a PowerPulse homogenizer.
4. Filter acetonitrile through a Buchner funnel into a 500-mL filter flask using vacuum and Whatman #4 filter paper.
5. Rinse the extraction jar and filter cake twice using 10-mL of deionized water each time.
6. Pour contents of filter flask into a 250-mL Erlenmeyer ground glass stoppered flask.

THE ANALYSIS CAN BE STOPPED AT THIS POINT AND THE STOPPERED SAMPLE EXTRACTS STORED FOR UP TO TWO DAYS AT 1 TO 8°C.

To continue with the analysis, add 5.0 grams of sodium chloride, and shake vigorously for 30 seconds. Allow the phases to separate for at least 30 minutes at which time an upper acetonitrile and lower water layer will be visible. Some undissolved sodium chloride may still remain on the bottom of the flask.

B. Acetonitrile/Hexane Partition

1. Remove a 20.0-mL aliquot of acetonitrile (representing 2.0-g sample) using a volumetric pipette to a 125-mL separatory funnel. Add 20 mL of hexane, and shake for 30 seconds. Drain the lower acetonitrile layer into a 150-mL beaker. Discard the upper hexane layer.

2. Transfer the acetonitrile back to the separatory funnel. Add 20 mL of hexane to the beaker, swirl and transfer to the separatory funnel. Shake for 30 seconds. Drain the lower acetonitrile layer back into a 150-mL beaker. Discard the upper hexane layer.
3. Repeat the partition once more using 20 mL of hexane. Drain the lower acetonitrile layer into a 100 mL clear Boston Round bottle with Teflon®-lined screw cap lid. Discard the upper hexane layer.

THE ANALYSIS CAN BE STOPPED AT THIS POINT AND THE STOPPERED SAMPLE EXTRACTS STORED FOR UP TO TWO DAYS AT 1 TO 8°C.

C. Florisil™ Cleanup

One cartridge from each new box of Florisil® Bond Elut LRC® cartridges must be fractionated as follows with a known quantity of the reference standard using control sample matrix.

[Fractionation: Perform step 1 of the Florisil® Clean-up using "clean" control sample matrix (i.e., evaporate a 5.0-mL aliquot of extract representing a 0.5-g sample). After taking the sample to dryness, fortify the dried control sample matrix at 0.2 ppm (100 µL of 1-µg/mL methomyl standard). Take sample back to dryness by removing fortification solvent using nitrogen. Continue at step 2 of the Florisil® Clean-up. Collect fractions from the cartridge following each step: load, hexane wash, 10:90 acetone:hexane wash, and 50:50 acetone:hexane elution. Submit each fraction to HPLC for analysis. Losses of no greater than 10% are generally acceptable.]

The elution volume and/or polarity of the suggested eluting mixture may need to be adjusted in order to obtain quantitative elutions.

1. Remove a 5-mL aliquot of acetonitrile (representing 0.5-g sample) using a volumetric pipette to a 13 x 100 mm test tube.
 - a. Steambath evaporation: Reduce solvent volume on a steambath to 0.1 - 0.2 mL. Manually take the sample to dryness (takes about 5 minutes/sample) using nitrogen. Do not overdry.

-or-

- b. N-EVAP evaporation: Reduce solvent volume on an N-EVAP maintained at 30-35°C. The nitrogen flow should be set so as to cause a 1-2 mm dent on the surface of the solution. Reduce solvent volume to 0.1 - 0.2 mL. Manually take the sample to dryness (takes about 5 minutes/sample) using nitrogen. Do not overdry.

THE ANALYSIS CAN BE STOPPED AT THIS POINT AND THE STOPPERED SAMPLE EXTRACTS STORED FOR UP TO TWO DAYS AT 1 TO 8°C.

2. Dissolve the residue in 1 mL of dichloromethane; vortex five seconds. See Note A.
3. Using the Vac Elut SPS 24™ Vacuum Manifold (use gravity flow, i.e., no vacuum), prewash the Florisil™ Bond Elut® LRC cartridge with 5 mL of hexane. See Note B. The manifold should be in the "waste" position.

Note A: Do not leave the sample residue in dichloromethane overnight as possible losses of methomyl may occur.

Note B: A vacuum is not used with the Vac Elut SPS 24™; gravity flow is used. There are two reasons for this:

Due to the nature of the solvents, even a slight vacuum causes the solvents to flow from the Bond Elut® cartridge in a continuous stream. Only through considerable manipulation of the stopcock will the desired flow rate be attained.

Although the desired flow rate can be obtained when running 2-3 samples at once, it is not possible to simultaneously run more than 3 samples without some Bond Elut® cartridges going dry. This is due to the small solvent volumes employed. By using gravity flow and the stopcocks, any number of samples can be processed simultaneously on the Vac Elut SPS 24™.

4. Load the sample in 1 mL of dichloromethane onto the cartridge using a disposable pasteur pipette. The manifold is in the "waste" position. See Note C.

Note C: The flow rate from the cartridge should not be a steady stream of liquid but a steady drip such that individual drops can be seen forming at the tip of the cartridge. For ease in controlling the drip rate, stopcocks can be attached to the cartridges.

5. Wash the 13- x 100-mm test tube with 5 mL of hexane. Transfer wash solution to the cartridge using a disposable pasteur pipette. The manifold is in the "waste" position.
6. Wash the 13- x 100-mm test tube with 7 mL of 10:90 acetone:hexane. Transfer wash solution to the cartridge using a disposable pasteur pipette. The manifold is in the "waste" position.
7. Wash the 13- x 100-mm test tube with 7 mL of 50:50 acetone:hexane. Transfer this solution to the cartridge using a disposable pasteur pipette. Elute the cartridge with the manifold in the "collect" position. Collect the eluate in pre-labeled 13- x 100-mm test tubes.
8. Add 5 drops of 1% decanol in acetone as a keeper to the eluate from #7 above and vortex for 30 seconds.

THE ANALYSIS CAN BE STOPPED AT THIS POINT AND THE STOPPERED SAMPLE EXTRACTS STORED FOR UP TO TWO DAYS AT 1 TO 8°C.

- a. Steambath evaporation: Reduce solvent volume on a steambath to 0.1 - 0.2 mL. Manually take the sample to dryness (takes about 5 minutes/sample) using nitrogen. Do not overdry.

-or-

- b. N-EVAP evaporation: Reduce solvent volume on an N-EVAP maintained at 30-35°C. The nitrogen flow should be set so as to cause a 1 - 2-mm dent on the surface of the solution. Reduce solvent volume to 0.1 - 0.2 mL. Manually take the sample to dryness (takes about 5 minutes/sample) using nitrogen. Do not overdry.
9. Add 1.0 mL of 15:85 acetonitrile:HPLC-grade water using a volumetric pipette; vortex for five seconds. Submit for HPLC analysis.

At this point 1.0 mL of solution represents 0.5 g of sample.

D. Chromatography

The HPLC system is described in the **EQUIPMENT** section.

Historically, the column and conditions stated in the method have been satisfactory for the matrix being analyzed. The specific column packing, mobile phase, column temperature, and flow rate listed are typical conditions for this analysis. Specific conditions for each HPLC run should be noted on each chromatogram and need not otherwise be documented.

1. Filter sample through a Millipore® HV 0.45- μ m filter directly into an autosampler vial at the time of HPLC analysis.
2. Detect and quantitate methomyl using postcolumn derivatization and Fluorescence detection. Typical conditions are:

Injection Volume: 50 μ L

Gradient:

(for pea seed, sorghum forage and hay, soybean hay,
and sugar beet foliage)

<u>Time (min)</u>	<u>% HPLC-Grade Water</u>	<u>% Acetonitrile</u>
0.0	95	5
15.0	60	40
18.0	60	40
19.0	95	5
22.0	95	5

Stop Time: 22.0 min

Gradient:

(for pea hay)

<u>Time (min)</u>	<u>% HPLC-Grade Water</u>	<u>% Acetonitrile</u>
0.0	95	5
15.0	75	25
17.0	25	75
19.0	25	75
20.0	95	5
24.0	95	5

Stop Time: 24.0 min

Column Temperature: 40°C

Flow Rate: 1.0 mL/min.

Post Column Conditions

Reaction Temperature: 100°C

Hydrolysis Reagent: 0.2 N NaOH

Derivatization Reagent: 500 mg OPA + approximately 2.5 g
N,N-dimethyl-2
mercaptoethylamine-
hydrochloride in 1 quart 0.05 M
sodium borate pH 9.1

Reagent Flow Rate: 0.13 mL/min each

Detector Settings: EX Wavelength: 330 nm
EM Wavelength: 466 nm

Radiolabelled Extraction Efficiency Validation

1. Ten raw agricultural commodity samples were used (2 dry pea seed, 2 dry pea hay, 2 sorghum fodder, 2 sorghum hay, and 2 sugar beet foliage).
2. Ten gram homogenized samples were weighed and transferred into individually labeled centrifuge bottles.
3. Radioactive methomyl (5.0 mg) was dissolved in 20 mL of acetonitrile. Three 10- μ L aliquots of the fortification solution were added to labeled scintillation vials. Ten mL of scintillation counting fluid was added to each aliquot. The aliquots were counted to determine the concentration of radioactivity in the fortification solution.
4. All samples were fortified with 1.0 mL (25 ppm) of radiolabelled methomyl and air dried for at least 24 hours.
5. Ten mL of distilled water was added to each sample and allowed to soak, without stirring, for 10 minutes.
6. One hundred milliliters of acetonitrile was added and blended at high speed for 2 minutes using a Tekmar[®] Tissumizer.
7. The extract was filtered through a Buchner funnel into a 500-mL filter flask using vacuum and Whatman #4 filter paper.
8. The extraction jar and filter cake were rinsed twice using 10 mL of deionized water each time. A small amount (~5 mL) of additional deionized water was used to rinse the extraction jar and filter cake a final time.
9. The final volume of each sample was recorded.
10. For all samples, three 1.0-mL aliquots were taken from each supernatant and added to labeled scintillation vials. Ten milliliters of scintillation counting fluid was added to

each aliquot of supernatant prior to the aliquots being analyzed to determine the concentration of radioactivity remaining.

Calculations

Concentration of Analyte in Sample (ppm found)

1. The methomyl concentration found in the HPLC injection solution or the mass of methomyl injected on-column (ng) is calculated by the integrator in the following way:

A four-point standard curve is constructed using peak heights of known standard injections versus the concentration (ng/mL) or mass on-column (ng) of the solutions injected. Linear least squares regression is applied to the curve to generate the standard curve equation: peak height = (slope) (concentration injected, ng/mL) + intercept. The methomyl concentration or mass on-column for each injection solution is calculated from the standard curve equation using the chromatographic peak height. (The standard curves are also manually plotted for visual inspection.)

2. Methomyl residue is reported in units of parts per million (ppm or μg methomyl/g sample). The following equation is used to calculate methomyl concentration in terms of the concentration (ng/mL) of methomyl injected per concentration (g/mL) of sample injected. (Note: If the integrator calculates nanograms methomyl on-column, divide by the injection volume (mL) to determine the solution concentration (ng/mL).

$$\text{methomyl concentration (ppm)} = \text{concentration injected (ng / mL)} \times \frac{1 \mu\text{g}}{1000 \text{ ng}} \times$$

$$\frac{1}{\text{initial sample weight (g)}} \times \frac{\text{extraction solvent volume (mL)}}{\text{SPE aliquot volume (mL)}} \times$$

$$\text{final volume (mL)} \times \text{dilution factor (if any)}$$

where

initial sample weight (g) = 10 g for crops analyzed

extraction solvent volume (mL) = total volume of solvent used for the extraction

- SPE aliquot volume (mL) = volume of extract for solid-phase clean-up
- final volume (mL) = standard reconstitution volume in the case of no dilution
- dilution factor (if any) = ratio of volume of diluted solution for injection to the volume of injection solution if dilution was not performed.
- concentration injected (ng/mL) = concentration of methomyl in injection solution; calculated from the calibration curve based on peak height as follows:

$$\text{concentration injected (ng / mL)} = \frac{\text{peak height} - \text{intercept}}{\text{slope}}$$

3. Report analytical result to two significant figures due to the degree of uncertainty at the low end of the concentration range.

Calculation of Fortification Recovery

- 1) Find parts per million in fortified sample as described above.
- 2) Use the following equation to calculate recovery:

$$\% \text{ Recovery} = \frac{\text{methomyl concentration (ppm)}}{\text{fortification level (ppm)}} \times 100 \%$$

where

fortification level (ppm) can be calculated as follows:

$$\text{fortification level (ppm)} = \frac{\text{volume fortification standard (mL)} \times \text{concentration fortification standard (mg / mL)}}{\text{sample weight (g)}}$$

- 3) Report % recovery to the nearest whole number.

Example Calculation

The calculations below show the methomyl concentration (ppm) in the sugar beet foliage sample (Sample No. S00099038, Spike B, fortified at 0.50 ppm), extracted on April 22, 1994 and analyzed on April 27, 1994. The peak height corresponded to 43.052 ng/mL of methomyl on the standard curve*.

$$\text{methomyl concentration (ppm)} = 0.43052 \text{ ppm} =$$

$$43.052 \text{ ng / mL} \times \frac{1 \mu\text{g}}{1000 \text{ ng}} \times \frac{1}{10.0 \text{ g}} \times \frac{100 \text{ mL}}{5.0 \text{ mL}} \times 1.0 \text{ mL} \times 5$$

$$\text{reported methomyl concentration (ppm)} = 0.43 \text{ ppm}$$

$$\% \text{ Recovery} = 86 \% = \frac{0.43 \text{ ppm}}{0.50 \text{ ppm}} \times 100 \%$$

RESULTS AND DISCUSSION**Recoveries**

Tables I-VI summarize the recovery data obtained from dry pea seed, dry pea hay, sorghum forage, sorghum hay, soybean hay, and sugar beet foliage fortified with methomyl.

Figures 1 and 2 show a typical calibration curve and the chromatograms of the calibration standards used to generate the curve. Typical chromatograms for the six matrices are shown in Figures 3-8.

* Concentration injected (ng/mL) is calculated by the integrator before rounding of the peak height, slope, and intercept values.

Accuracy and precision data for fortification recoveries for all six matrices are summarized below.

<u>Crop Matrix</u>	<u>Number of Fortifications</u>	<u>Range of Recoveries</u>	<u>Mean % Recovery ± Standard Deviation</u>	<u>Reference</u>
pea seed	9	75-90%	83% ± 5%	2
pea hay	9	82-100%	88% ± 5%	2
sorghum forage	9	75-105%	90% ± 9%	this study
sorghum hay	9	76-100%	90% ± 8%	this study
soybean hay	16	85-145%*	98% ± 14%	4
sugar beet foliage	21	79-100%	87% ± 7%	3

Limit of Quantitation

The limit of quantitation (LOQ) for this method is 0.020-ppm methomyl in dry pea seed and hay, sorghum forage and hay, soybean hay, and sugar beet foliage. The LOQ is defined by (1) a peak height of approximately 10 times the baseline noise, and (2) the ability to reliably recover 70-120 % of the methomyl from a sample fortified at the limit of quantitation. The limit of quantitation in all matrices are affected by matrix interferences.

¹⁴C Extraction Efficiency Validation

To demonstrate the validity of the extraction procedure discussed in this method, the extraction efficiency was studied in pea seed and hay, sorghum fodder[†] and hay, and sugar beet foliage using radiolabelled methomyl. The procedure is described in the experimental section as Radiolabelled Extraction Efficiency Validation (these steps correspond to Section A, steps 1-5 of the method).

* Recoveries were not corrected for methomyl detected in the control samples. Two high recoveries (135% and 145%) were reported because some of the controls from the magnitude of residue study contained methomyl.

† Sorghum fodder was substituted for sorghum forage in the efficiency study due to availability. Because sorghum fodder is more dry than sorghum forage, methomyl ought to be more difficult to extract from fodder than forage. Therefore, acceptable extraction efficiency on sorghum fodder, indicates that the extraction will work equally well on sorghum forage.

The results for each matrix are summarized in Table VII. Acceptable extraction efficiency was demonstrated (average extraction efficiency was $103\% \pm 3\%$) indicating that extraction with acetonitrile followed by two deionized water washes was sufficient to extract methomyl from pea seed and hay, sorghum fodder and hay, and sugar beet foliage.

The raw data and calculations are shown in Appendix II.

Time Required for Analysis

The time required for sample analysis is dependent upon the number of samples being analyzed at one time. For a sample set of 10 samples (treated samples plus quality control samples), nine working hours are required to take the samples through method steps A (not including sample weighing), B and C. Using an autosampler, the HPLC can be performed overnight.

Interference Check

The following carbamates were chromatographed under the conditions described in this method to check for peak interference with methomyl: oxamyl, carbaryl, thiodicarb, aldicarb, and carbofuran. Injection of the thiodicarb standard did result in a small methomyl peak in addition to the thiodicarb peak. Since thiodicarb breaks down to produce methomyl, this result was expected. The retention times for each carbamate is shown below. As can be seen, methomyl was separated from the five other carbamates.

Gradient for pea seed, sorghum forage and hay, soybean hay, and sugar beet foliage:

<u>Carbamate</u>	<u>Retention Time (min)</u>
Methomyl	17.0
Oxamyl	16.5
Aldicarb	23.8
Thiodicarb	27.1
Carbofuran	27.3
Carbaryl	34.9*

* In order to elute carbaryl, the percent acetonitrile was increased to 40% following the 22-minute stop time.

Gradient for pea hay** :

<u>Carbamate</u>	<u>Retention Time (min)</u>
Methomyl	19.1
Oxamyl	18.7
Aldicarb	25.5
Thiodicarb	26.2
Carbofuran	26.4
Carbaryl	26.5

Confirmatory Technique

Methomyl-fortified sorghum forage and hay samples were extracted and prepared for analysis as per Steps A-C of this method. The extracts were analyzed by (1) the HPLC/post-column derivatization method described here, and (2) thermospray liquid chromatography tandem mass spectrometry (LC/MS/MS) using the instrumentation and conditions listed in Appendix III.

The tandem mass spectrometry technique monitored two product ions which were formed by colliding the protonated molecular ion with argon gas. This technique provides a highly specific confirmation of methomyl. Representative raw data showing a total ion chromatogram with the two product ion scans is presented in Appendix III.

A summary of the recovery data for both methods is presented in Table VIII. For six sorghum forage samples and six sorghum hay samples, the recoveries using LC/MS/MS detection range from 92-106% and 86-122% with mean recoveries of $98\% \pm 5\%$ and $103\% \pm 12\%$. These data indicate that LC/MS/MS provides both qualitative and quantitative confirmatory technique for the methomyl analysis.

** In order to elute aldicarb, thiodicarb, carbofuran, and carbaryl, the percent acetonitrile was increased to 25% following the 24-minute stop time.

CONCLUSIONS

This analytical method is suitable for the determination of methomyl in dry pea seed and hay, sorghum forage and hay, soybean hay, and sugar beet foliage at levels down to 0.020 ppm.

ACKNOWLEDGEMENTS

The quality data produced by Robert M. Henze and Kimberly O. Fisher of DuPont Agricultural Products, and Robert Bethem of Alta Analytical Laboratories, Inc., is gratefully acknowledged.

CERTIFICATION

**ENFORCEMENT METHOD FOR THE DETERMINATION OF
METHOMYL IN DRY PEA SEED AND HAY, SORGHUM
FORAGE AND HAY, SOYBEAN HAY, AND SUGAR BEET
FOLIAGE**

We, the undersigned, declare that this report provides an accurate record of the procedures and results.

Report by:

Janet C. Rühl
Janet C. Rühl
Study Director

13-OCT-94
Date

Approved by:

Jeanette M. Erhardt
Jeanette M. Erhardt
Research Manager

13 Oct 1994
Date

Date Study Completed:

October 13, 1994

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DuPont Agricultural Products
Global Technology Division
Experimental Station
Wilmington, Delaware 19880-0402

TABLE I
RECOVERY OF METHOMYL FROM PEA SEED¹

<u>Fortification Level (ppm)</u>	<u>ppm Methomyl Found</u>	<u>% Recovery</u>
0.020	0.017	85
0.020	0.017	85
0.020	0.017	85
0.20	0.15	75
0.20	0.16	80
0.20	0.18	
reinjection	0.18	90*
2.0	1.6	80
2.0	1.6	80
Mean % Recovery: 82 % \pm 5 % (s.d.)		
Range: 75 - 90 %		

* Average recovery for two injections.

¹ DuPont Agricultural Products AMR 2566-93, "Magnitude of Residues of Methomyl in Field Peas Following Application of Lannate® Insecticide at Maximum Label Rates", authored by D. M. Tomic.

TABLE II
RECOVERY OF METHOMYL FROM PEA HAY¹

<u>Fortification Level (ppm)</u>	<u>ppm Methomyl Found</u>	<u>% Recovery</u>
0.020	0.018	90
0.020	0.018	90
0.020	0.020	100
0.50	0.42	84
0.50	0.41	82
0.50	0.44	
reinjection	0.42	86*
2.0	1.8	90
2.0	1.7	85
Mean % Recovery: 88 % \pm 6 % (s.d.)		
Range: 82 -100 %		

* Average recovery for two injections.

¹ DuPont Agricultural Products AMR 2566-93, "Magnitude of Residues of Methomyl in Field Peas Following Application of Lannate® Insecticide at Maximum Label Rates", authored by D. M. Tomic.

TABLE III
RECOVERY OF METHOMYL FROM SORGHUM FORAGE

<u>Fortification Level (ppm)</u>	<u>ppm Methomyl Found</u>	<u>% Recovery</u>
0.020	0.021	105
0.020	0.020	100
0.020	0.017	85
0.10	0.097	97
0.10	0.083	83
0.10	0.075	75
1.0	0.92	92
1.0	0.92	92
1.0	0.85	85
Mean % Recovery: 90 % \pm 9 % (s.d.)		
Range: 75 - 105 %		

TABLE IV
RECOVERY OF METHOMYL FROM SORGHUM HAY

<u>Fortification Level (ppm)</u>	<u>ppm Methomyl Found</u>	<u>% Recovery</u>
0.020	0.020	100
0.020	0.019	95
0.020	0.019	95
0.10	0.076	76
0.10	0.092	92
0.10	0.092	92
1.0	0.89	89
1.0	0.80	80
1.0	0.91	91

Mean % Recovery: 90 ± 8 % (s.d.)

Range: 76 - 100 %

TABLE V
RECOVERY OF METHOMYL FROM SOYBEAN HAY¹

<u>Fortification Level (ppm)</u>	<u>ppm Methomyl Found</u>	<u>% Recovery</u>
0.020	0.018	90
0.020	0.018	90
0.020	0.017	85
0.020	0.019	95
0.020	0.019	95
0.020	0.019	95
0.020	0.019	95
0.020	0.029	145
0.050	0.050	100
0.050	0.049	98
0.10	0.092	92
0.10	0.093	93
0.10	0.086	86
0.10	0.090	90
0.20	0.19	95
0.20	0.19	95
0.20	0.27	135
0.50	0.49	98
1.0	0.98	98
1.0	0.92	92
1.0	1.1	110
10.0	9.4	94

Mean % Recovery: 88 % \pm 14 % (s.d.)

Range: 85 - 145 %

¹ DuPont Agricultural Products AMR 2650-93, "Magnitude of Residues of Methomyl in Soybean Hay Following Application of Lannate® Insecticide at Maximum Label Rates", authored by J. C. Rühl.

TABLE VI
RECOVERY OF METHOMYL FROM SUGAR BEET FOLIAGE¹

<u>Fortification Level (ppm)</u>	<u>ppm Methomyl Found</u>	<u>% Recovery</u>
0.020	0.020	100
0.020	0.020	100
0.020	0.019	95
0.020	0.017	85
0.020	0.018	90
0.10	0.087	87
0.20	0.17	85
0.20	0.17	85
0.20	0.17	85
0.50	0.43	86
0.50	0.45	90
1.00	0.84	84
1.00	0.68	68
1.00	0.88	88
1.00	0.88	88
1.00	0.79	79
1.00	0.91	91
1.00	0.81	81
1.00	0.88	88
2.00	1.7	85
3.00	2.9	97

Mean % Recovery: 87% \pm 7% (s.d.)

Range: 68-100%

¹ DuPont Agricultural Products AMR 2653-93, "Magnitude of Residues of Methomyl in Sugar Beet Foliage Following Application of Lannate® LV Insecticide", authored by J. C. Rühl.

TABLE VII
SUMMARY OF EXTRACTION EFFICIENCY RESULTS

Matrix	Percent Extraction Efficiency	
	Replicate 1 Replicate 2	Average for Matrix
Dry Pea Hay	99	100
	102	
Dry Pea Seed	108	108
	107	
Sorghum Fodder	103	102
	102	
Sorghum Hay	101	100
	100	
Sugar beet Foliage	103	104
	105	

Overall average % extraction efficiency = 103 % \pm 3 %

TABLE VIII
CONFIRMATION OF METHOMYL RECOVERY
FROM SORGHUM FORAGE AND HAY

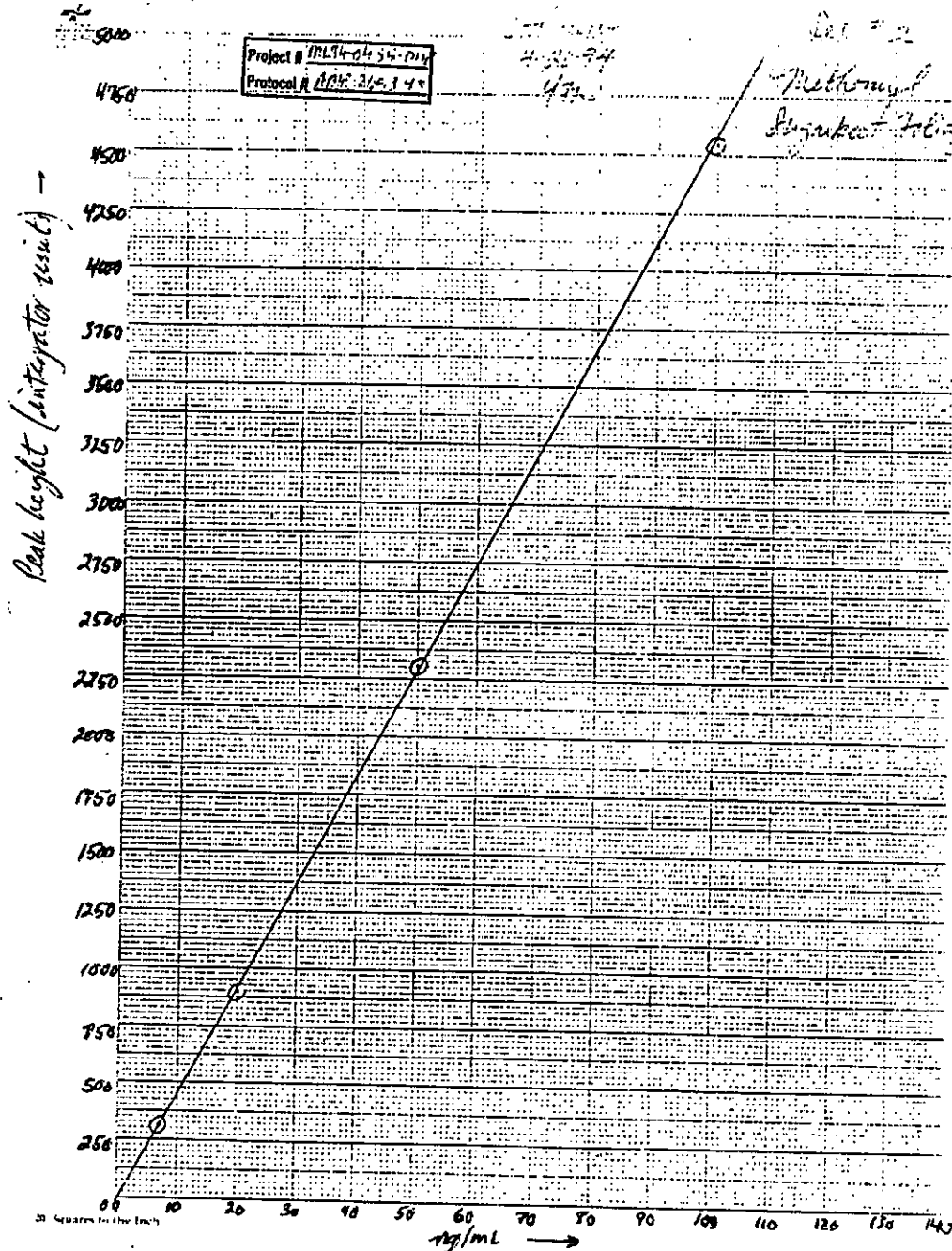
Matrix	Fortification Level (ppm)	% Recovery	
		AMR 3015-94 [*]	LC/MS/MS [†]
Sorghum Forage	0.020	95	98
	0.020	95	106
	0.10	87	95
	0.10	89	93
	1.0	90	92
	1.0	88	101
Average % Recovery = 91% ± 3% (s.d.)		98 % ± 5 % (s.d.)	

Matrix	Fortification Level (ppm)	% Recovery	
		AMR 3015-94 [*]	LC/MS/MS [†]
Sorghum Hay	0.020	90	106
	0.020	90	97
	0.10	86	94
	0.10	82	86
	1.0	86	114
	1.0	82	122
Average % Recovery = 86 % ± 3 % (s.d.)		103 % ± 12 % (s.d.)	

* DuPont Agricultural Products AMR 2343-92, "Magnitude of Residues of Methomyl in Sorghum Forage and Hay Following Multiple Application of Lannate® L Insecticide", Supplement No. 1, authored by J. C. Rühl.

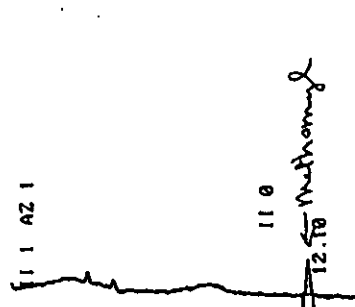
† Extracts analyzed using the instrumentation and conditions listed in Appendix III.

FIGURE 1
CALIBRATION CURVE FOR METHOMYL STANDARDS¹
(65% OF ORIGINAL SIZE)

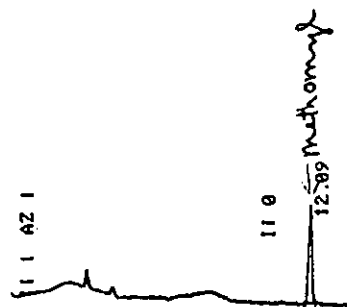


¹ DuPont Agricultural Products AMR 2653-93, "Magnitude of Residues of Methomyl in Sugarbeet Foliage Following Application of Lannate® LV Insecticide", authored by J. C. Rühl.

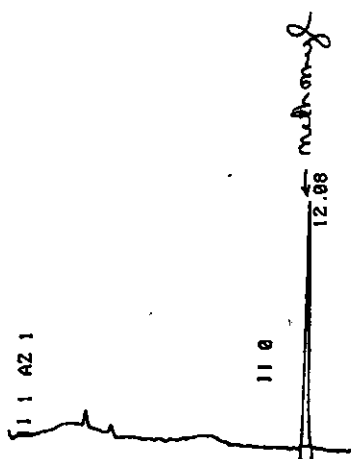
FIGURE 2
CHROMATOGRAMS OF METHOMYL STANDARDS¹
(65% OF ORIGINAL SIZE)



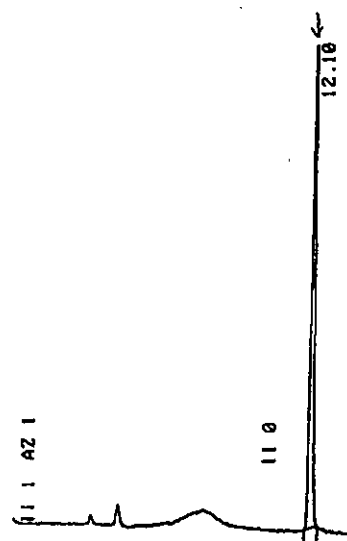
7-ng/mL Methomyl Standard



20-ng/mL Methomyl Standard



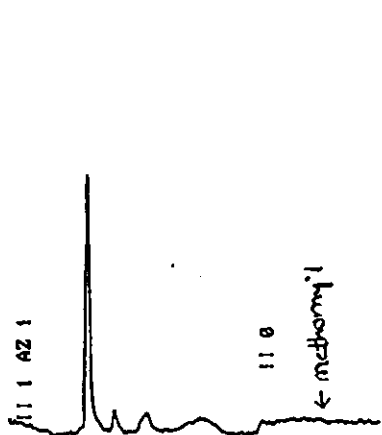
50-ng/mL Methomyl Standard



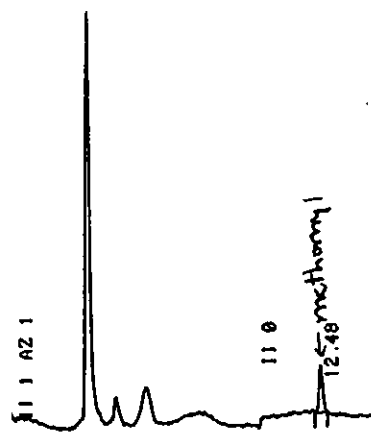
100-ng/mL Methomyl Standard

¹ DuPont Agricultural Products AMR 2653-93, "Magnitude of Residues of Methomyl in Sugarbeet Foliage Following Application of Lannate® LV Insecticide", authored by J. C. Rühl.

FIGURE 3
CHROMATOGRAMS OF CONTROL AND FORTIFIED DRY PEA SEED¹
(65% OF ORIGINAL SIZE)



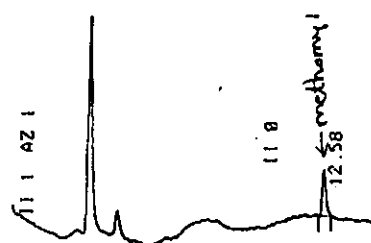
Analytical Set No.: 1
 Sample No: S00105218 CK
 Fortified at: Control
 Dilution Factor: 1
 Recovery: Control Sample



Analytical Set No.: 1
 Sample No: S00105218 SPK1
 Fortified at: 0.020 ppm
 Dilution Factor: 1
 Recovery: 85%

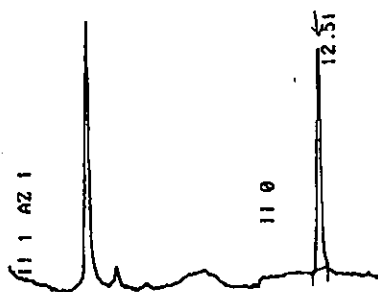


Analytical Set No.: 1
 Sample No: S00105218SPK1 DUP
 Fortified at: 0.020 ppm
 Dilution Factor: 1
 Recovery: 85%



Analytical Set No.: 4
 Sample No: S00105218 SPK A
 Fortified at: 0.020ppm
 Dilution Factor: 1
 Recovery: 85%

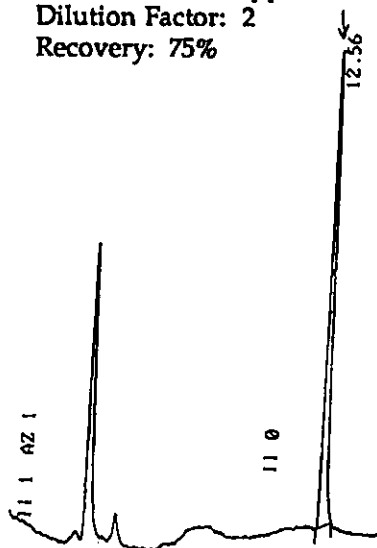
¹ DuPont Agricultural Products AMR 2566-93, "Magnitude of Residues of Methomyl in Pea Seed and Hay Following Application of Lannate® LV Insecticide", authored by D. M. Tomic.

FIGURE 3 (CONTINUED)

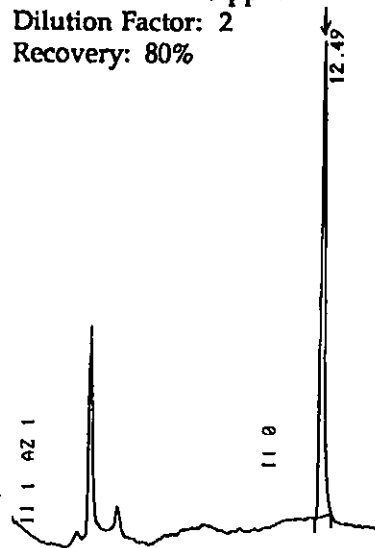
Analytical Set No.: 1
Sample No: S00105218 SPK2
Fortified at: 0.20 ppm
Dilution Factor: 2
Recovery: 75%



Analytical Set No.: 1
Sample No: S00105218 SPK2 DUP
Fortified at: 0.20 ppm
Dilution Factor: 2
Recovery: 80%



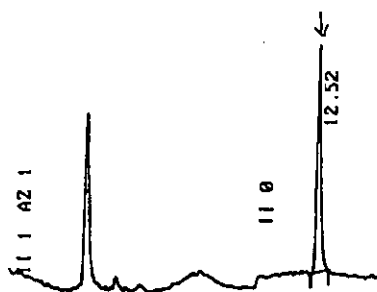
Analytical Set No.: 4
Sample No: S00105218 SPK B
Fortified at: 0.20 ppm
Dilution Factor: 1
Recovery: 90%



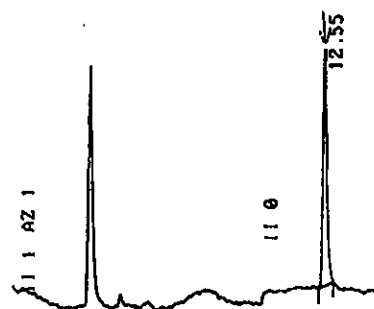
Analytical Set No.: 4R
Sample No: S00105218 SPK B
Fortified at: 0.20ppm
Dilution Factor: 1
Recovery: 90%

¹ DuPont Agricultural Products AMR 2566-93, "Magnitude of Residues of Methomyl in Pea Seed and Hay Following Application of Lannate® LV Insecticide", authored by D. M. Tomic.

FIGURE 3 (CONTINUED)



Analytical Set No.: 1
 Sample No: S00105218 SPK3
 Fortified at: 2.0 ppm
 Dilution Factor: 20
 Recovery: 80%



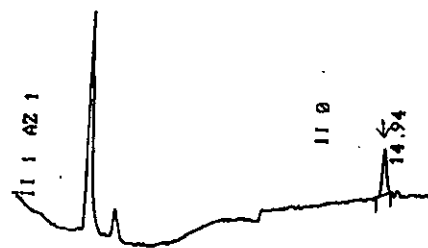
Analytical Set No.: 1
 Sample No: S00105218 SPK3 DUP
 Fortified at: 2.0 ppm
 Dilution Factor: 20
 Recovery: 80%

¹ DuPont Agricultural Products AMR 2566-93, "Magnitude of Residues of Methomyl in Pea Seed and Hay Following Application of Lannate® LV Insecticide", authored by D. M. Tomic.

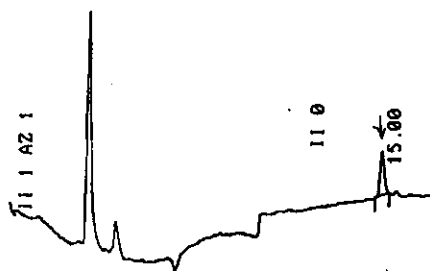
FIGURE 4
CHROMATOGRAMS OF CONTROL AND FORTIFIED DRY PEA HAY¹
(65% OF ORIGINAL SIZE)



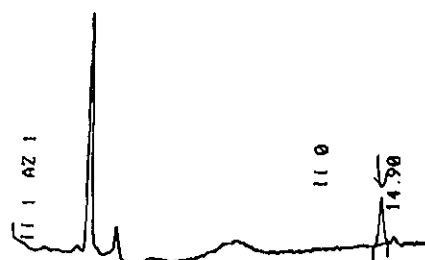
Analytical Set No.: 2
 Sample No: S00105203 CK
 Fortified at: Control
 Dilution Factor: 1
 Recovery: Control Sample



Analytical Set No.: 2
 Sample No: S00105203 SPK1
 Fortified at: 0.020 ppm
 Dilution Factor: 1
 Recovery: 90%



Analytical Set No.: 2
 Sample No: S00105203 SPK1 DUP
 Fortified at: 0.020 ppm
 Dilution Factor: 1
 Recovery: 90%



Analytical Set No.: 3
 Sample No: S00105203 SPK A
 Fortified at: 0.020 ppm
 Dilution Factor: 1
 Recovery: 100%

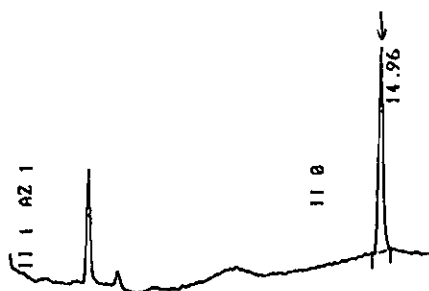
¹ DuPont Agricultural Products AMR 2566-93, "Magnitude of Residues of Methomyl in Pea Seed and Hay Following Application of Lannate® LV Insecticide", authored by D. M. Tomic.

FIGURE 4 (CONTINUED)

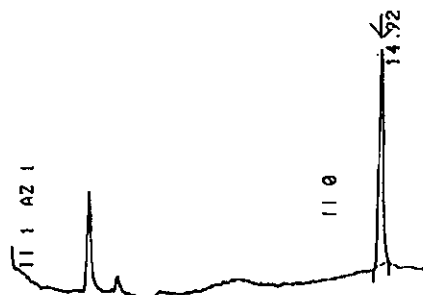
Analytical Set No.: 2
Sample No: S00105203 SPK2
Fortified at: 0.50 ppm
Dilution Factor: 5
Recovery: 84%



Analytical Set No.: 2
Sample No: S00105203 SPK2 DUP
Fortified at: 0.50 ppm
Dilution Factor: 5
Recovery: 82%



Analytical Set No.: 3
Sample No: S00105203 SPK B
Fortified at: 0.50 ppm
Dilution Factor: 5
Recovery: 88%



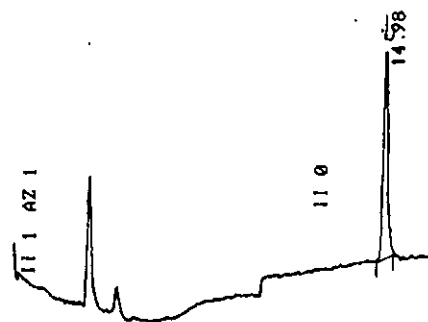
Analytical Set No.: 3R
Sample No: S00105203 SPK B
Fortified at: 0.50ppm
Dilution Factor: 5
Recovery: 84%

1. DuPont Agricultural Products AMR 2566-93, "Magnitude of Residues of Methomyl in Pea Seed and Hay Following Application of Lannate® LV Insecticide", authored by D. M. Tomic.

FIGURE 4 (CONTINUED)



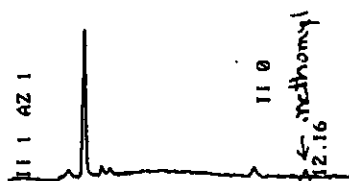
Analytical Set No.: 2
Sample No: S00105203 SPK3
Fortified at: 2.0 ppm
Dilution Factor: 20
Recovery: 90%



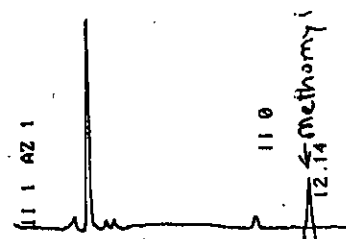
Analytical Set No.: 2
Sample No: S00105203 SPK3 DUP
Fortified at: 2.0 ppm
Dilution Factor: 20
Recovery: 85%

¹ DuPont Agricultural Products AMR 2566-93, "Magnitude of Residues of Methomyl in Pea Seed and Hay Following Application of Lannate® LV Insecticide", authored by D. M. Tomic.

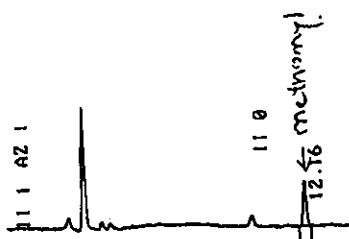
FIGURE 5
CHROMATOGRAMS OF CONTROL AND FORTIFIED SORGHUM FORAGE
(65% OF ORIGINAL SIZE)



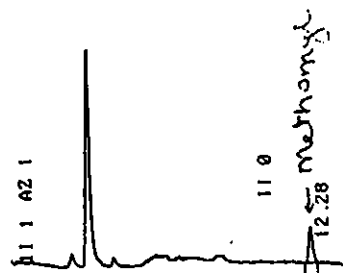
Analytical Set No.: 1
Sample No: S00035955 CK
Fortified at: control
Dilution Factor: 1
Recovery: control sample



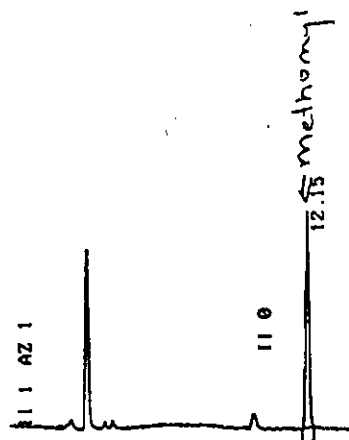
Analytical Set No.: 1
Sample No: S00035955 SPK A
Fortified at: 0.020 ppm
Dilution Factor: 1
Recovery: 92%



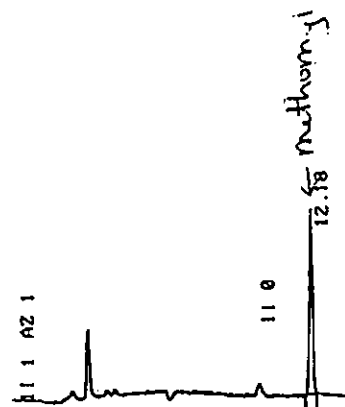
Analytical Set No.: 1
Sample No: S00035955 SPK A DUP
Fortified at: 0.020 ppm
Dilution Factor: 1
Recovery: 87%



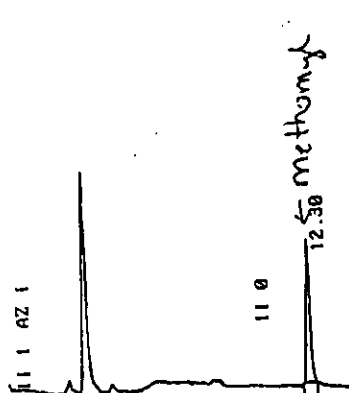
Analytical Set No.: 3
Sample No: S00035955 SPK A
Fortified at: 0.020ppm
Dilution Factor: 1
Recovery: 85%

FIGURE 5 (CONTINUED)

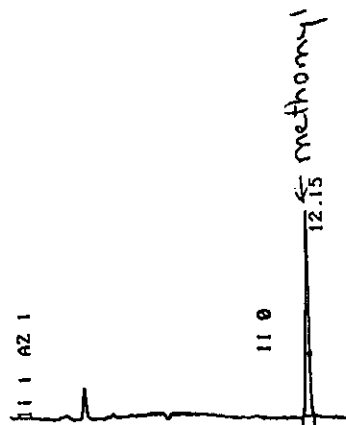
Analytical Set No.: 1
Sample No: S00035955 SPK B
Fortified at: 0.10 ppm
Dilution Factor: 1
Recovery: 94%



Analytical Set No.: 1
Sample No: S00035955 SPK B DUP
Fortified at: 0.10 ppm
Dilution Factor: 1
Recovery: 80%

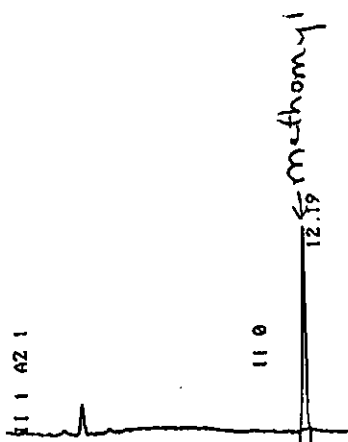


Analytical Set No.: 3
Sample No: S00035955 SPK B
Fortified at: 0.10 ppm
Dilution Factor: 1
Recovery: 75%

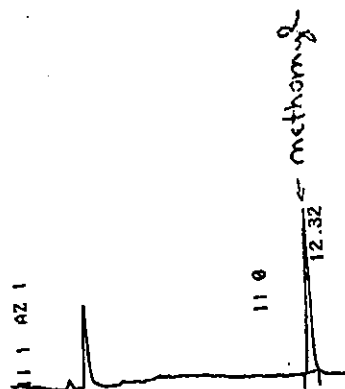


Analytical Set No.: 1
Sample No: S00035955 SPK C
Fortified at: 1.0 ppm
Dilution Factor: 10
Recovery: 92%

FIGURE 5 (CONTINUED)

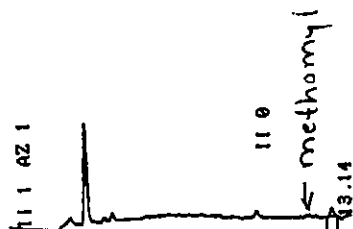


Analytical Set No.: 1
 Sample No: S00035955 SPK C DUP
 Fortified at: 1.0 ppm
 Dilution Factor: 10
 Recovery: 92%

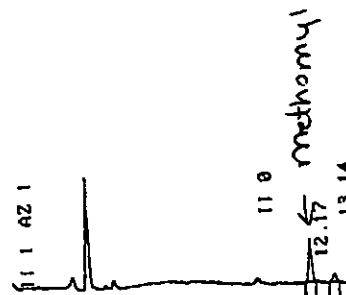


Analytical Set No.: 3
 Sample No: S00035955 SPK C
 Fortified at: 1.0 ppm
 Dilution Factor: 10
 Recovery: 85%

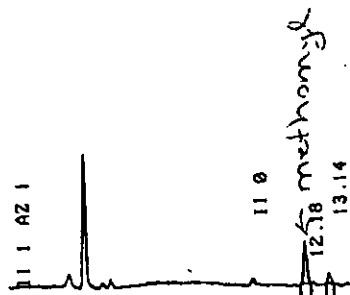
FIGURE 6
CHROMATOGRAMS OF CONTROL AND FORTIFIED SORGHUM HAY
(65% OF ORIGINAL SIZE)



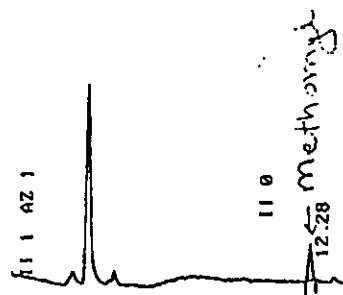
Analytical Set No.: 2
Sample No: S00032951 CK
Fortified at: control
Dilution Factor: 1
Recovery: control sample



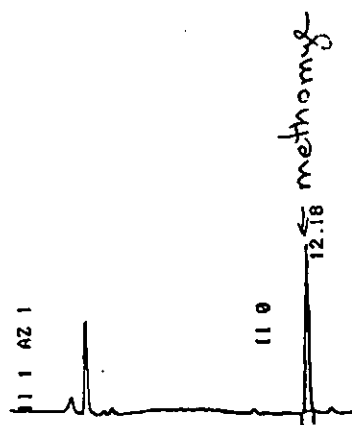
Analytical Set No.: 2
Sample No: S00032951 SPK A
Fortified at: 0.020 ppm
Dilution Factor: 1
Recovery: 100%



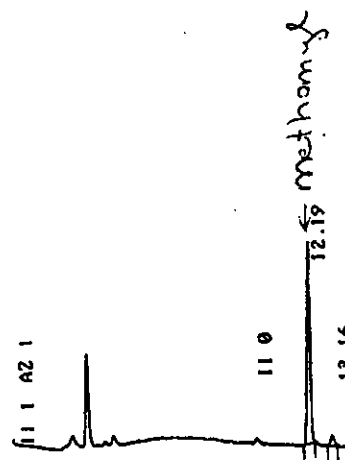
Analytical Set No.: 2
Sample No: S00032951 SPK A DUP
Fortified at: 0.020 ppm
Dilution Factor: 1
Recovery: 95%



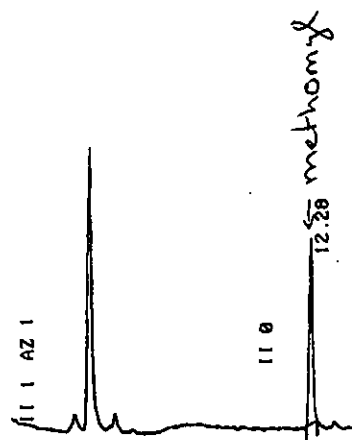
Analytical Set No.: 4
Sample No: S00032951 SPK A
Fortified at: 0.020ppm
Dilution Factor: 1
Recovery: 95%

FIGURE 6 (CONTINUED)

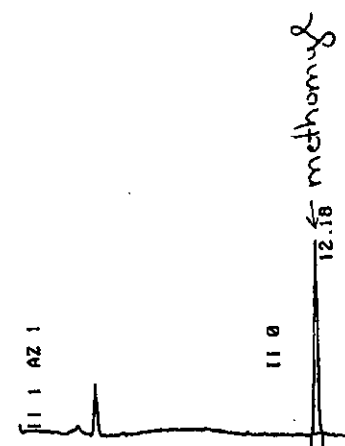
Analytical Set No.: 2
Sample No: S00032951 SPK B
Fortified at: 0.10 ppm
Dilution Factor: 1
Recovery: 76%



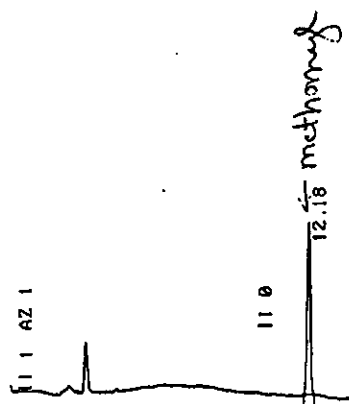
Analytical Set No.: 2
Sample No: S00032951 SPK B DUP
Fortified at: 0.10 ppm
Dilution Factor: 1
Recovery: 92%



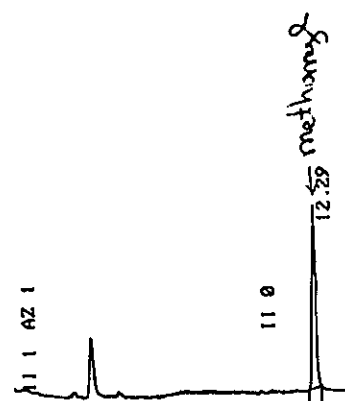
Analytical Set No.: 4
Sample No: S00032951 SPK B
Fortified at: 0.10 ppm
Dilution Factor: 1
Recovery: 92%



Analytical Set No.: 2
Sample No: S00032951 SPK C
Fortified at: 1.0 ppm
Dilution Factor: 10
Recovery: 89%

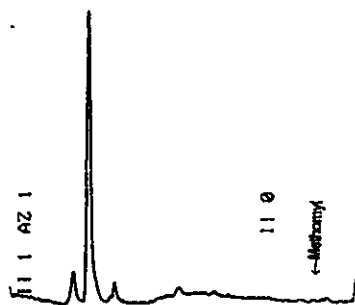
FIGURE 6 (CONTINUED)

Analytical Set No.: 2
Sample No: S00032951 SPK C DUP
Fortified at: 1.0 ppm
Dilution Factor: 10
Recovery: 80%

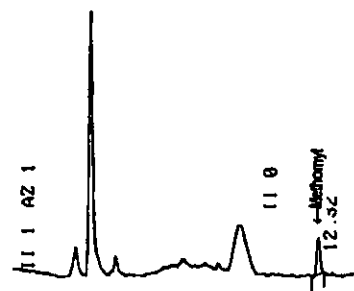


Analytical Set No.: 4
Sample No: S00032951 SPK C
Fortified at: 1.0 ppm
Dilution Factor: 10
Recovery: 91%

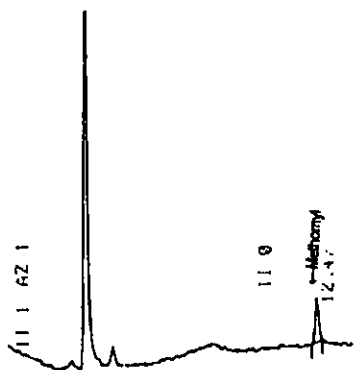
FIGURE 7
CHROMATOGRAMS OF CONTROL AND FORTIFIED SOYBEAN HAY¹
(65% OF ORIGINAL SIZE)



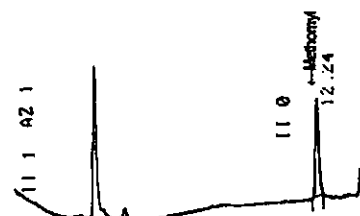
Analytical Set No.: 4
 Sample No: S00049975 CK
 Fortified at: Control
 Dilution Factor: 1
 Recovery: Control Sample



Analytical Set No.: 4
 Sample No: S00049975 SPK A
 Fortified at: 0.020 ppm
 Dilution Factor: 1
 Recovery: 95%

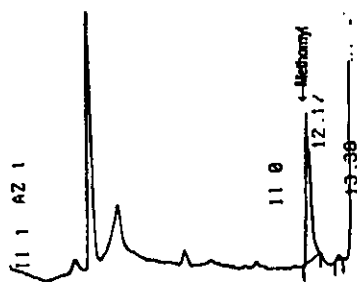


Analytical Set No.: 7
 Sample No: S00082012 SPK A
 Fortified at: 0.020 ppm
 Dilution Factor: 1
 Recovery: 95%

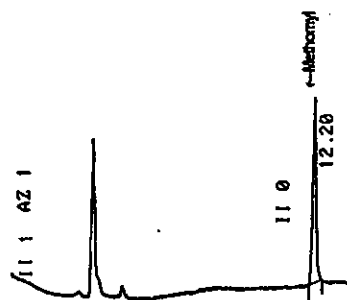


Analytical Set No.: 6
 Sample No: S00182024 SPK A
 Fortified at: 0.050 ppm
 Dilution Factor: 1
 Recovery: 100%

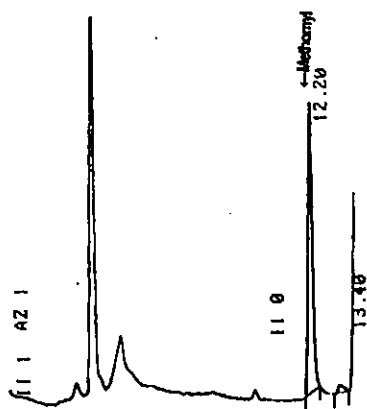
¹ DuPont Agricultural Products AMR 2650-93, "Magnitude of Residues of Methomyl in Soybean Hay Following Application of Lannate® Insecticide at Maximum Label Rates", authored by J. C. Rühl.

FIGURE 7 (CONTINUED)

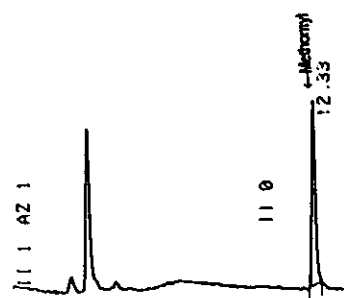
Analytical Set No.: 9
 Sample No: S00127182 SPK B
 Fortified at: 0.050 ppm
 Dilution Factor: 1
 Recovery: 98%



Analytical Set No.: 5
 Sample No: S00182016 SPK B
 Fortified at: 0.10 ppm
 Dilution Factor: 1
 Recovery: 92%

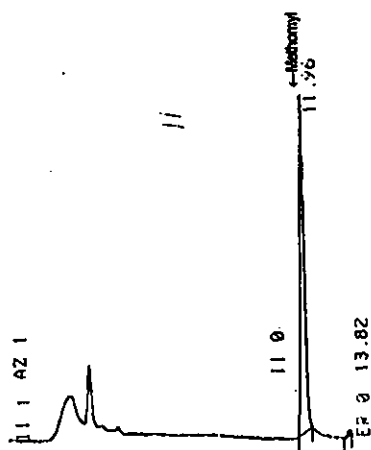


Analytical Set No.: 8
 Sample No: S00036643 SPK B
 Fortified at: 0.10 ppm
 Dilution Factor: 1
 Recovery: 90%

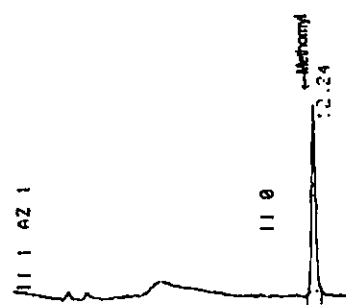


Analytical Set No.: 4
 Sample No: S00049975 SPK B
 Fortified at: 0.50 ppm
 Dilution Factor: 5
 Recovery: 98%

¹ DuPont Agricultural Products AMR 2650-93, "Magnitude of Residues of Methomyl in Soybean Hay Following Application of Lannate® Insecticide at Maximum Label Rates", authored by J. C. Rühl.

FIGURE 7 (CONTINUED)

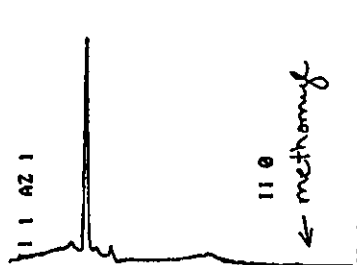
Analytical Set No.: 3
Sample No: S00100032 SPK B
Fortified at: 1.0 ppm
Dilution Factor: 10
Recovery: 110%



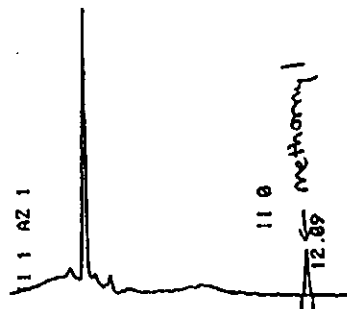
Analytical Set No.: 2
Sample No: S00095117 SPK B
Fortified at: 10.0 ppm
Dilution Factor: 100
Recovery: 94%

¹ DuPont Agricultural Products AMR 2650-93, "Magnitude of Residues of Methomyl in Soybean Hay Following Application of Lannate® Insecticide at Maximum Label Rates", authored by J. C. Rühl.

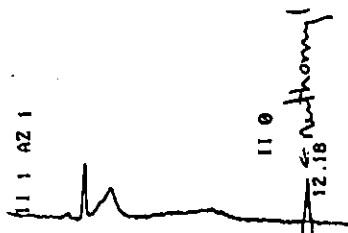
FIGURE 8
CHROMATOGRAMS OF CONTROL AND FORTIFIED SUGAR BEET FOLIAGE¹
(65% OF ORIGINAL SIZE)



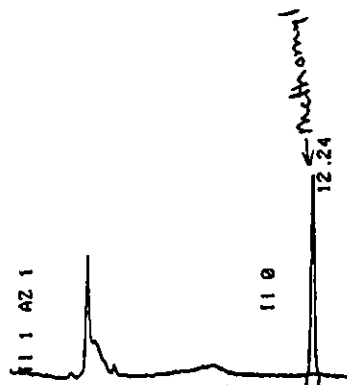
Analytical Set No.: 2
 Sample No: S00062348 CK
 Fortified at: Control
 Dilution Factor: 1
 Recovery: Control Sample



Analytical Set No.: 2
 Sample No: S00062348 SPK A
 Fortified at: 0.020 ppm
 Dilution Factor: 1
 Recovery: 95%

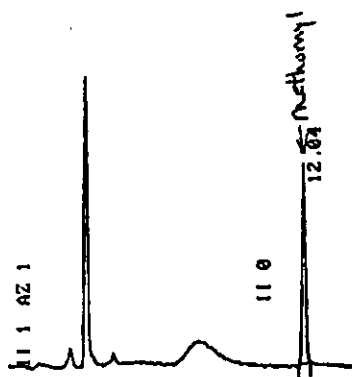


Analytical Set No.: 4
 Sample No: S00099038 SPK A
 Fortified at: 0.020 ppm
 Dilution Factor: 1
 Recovery: 90%

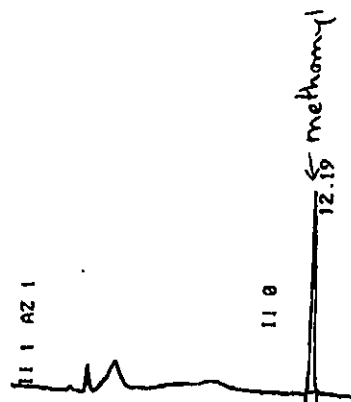


Analytical Set No.: 5
 Sample No: S00078131 SPK A
 Fortified at: 0.10 ppm
 Dilution Factor: 1
 Recovery: 87%

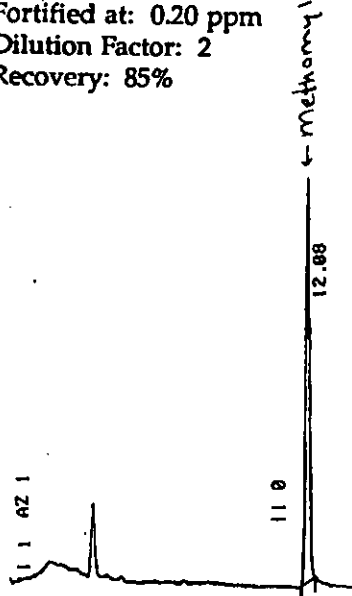
¹ DuPont Agricultural Products AMR 2653-93, "Magnitude of Residues of Methomyl in Sugarbeet Foliage Following Application of Lannate® LV Insecticide", authored by J. C. Rühl.

FIGURE 8 (CONTINUED)

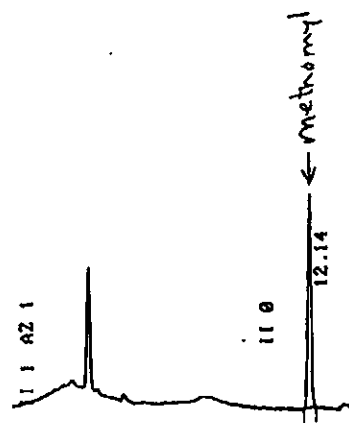
Analytical Set No.: 3
 Sample No: S00062356 SPK B
 Fortified at: 0.20 ppm
 Dilution Factor: 2
 Recovery: 85%



Analytical Set No.: 4
 Sample No: S00099038 SPK B
 Fortified at: 0.50 ppm
 Dilution Factor: 5
 Recovery: 86%



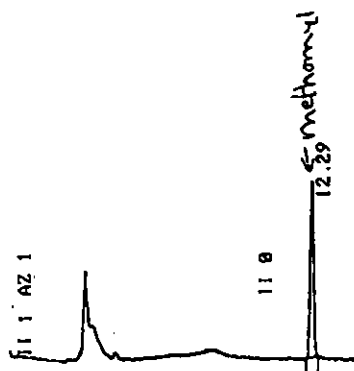
Analytical Set No.: 6
 Sample No: S00182008 SPK A
 Fortified at: 0.50 ppm
 Dilution Factor: 4
 Recovery: 90%



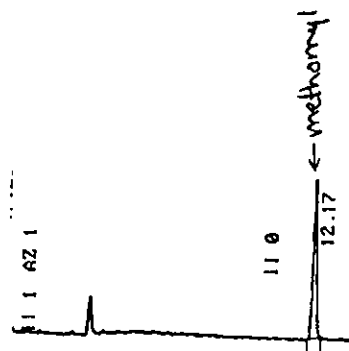
Analytical Set No.: 2
 Sample No: S00062348 SPK B
 Fortified at: 1.0 ppm
 Dilution Factor: 10
 Recovery: 88%

¹ DuPont Agricultural Products AMR 2653-93, "Magnitude of Residues of Methomyl in Sugarbeet Foliage Following Application of Lannate® LV Insecticide", authored by J. C. Rühl.

FIGURE 8 (CONTINUED)



Analytical Set No.: 5
 Sample No: S0078131 SPK B
 Fortified at: 1.0 ppm
 Dilution Factor: 10
 Recovery: 79%



Analytical Set No.: 7RA
 Sample No: S00078131 SPK B
 Fortified at: 2.0 ppm
 Dilution Factor: 20
 Recovery: 85%

¹ DuPont Agricultural Products AMR 2653-93, "Magnitude of Residues of Methomyl in Sugarbeet Foliage Following Application of Lannate® LV Insecticide", authored by J. C. Rühl.

REFERENCES

1. Clark, S., and Kennedy, S. M., "Analytical Method for the Quantitation of Methomyl in Grapes", DuPont Agricultural Products, AMR 1806-90, MRID No. 41721502.
2. Tomic, D. M., "Magnitude of Residues of Methomyl in Pea Seed and Hay Following Application of Lannate® LV Insecticide", DuPont Agricultural Products, AMR 2566-93, in progress.
3. Rühl, J. C. and Devine, P. G., "Magnitude of Residues of Methomyl in Sugarbeet Foliage Following Application of Lannate® LV Insecticide", DuPont Agricultural Products, AMR 2653-93, MRID No. 43346801.
4. Rühl, J. C. and Devine, P. G., "Magnitude of Residues of Methomyl in Soybean Hay Following Application of Lannate® Insecticide at Maximum Label Rates", DuPont Agricultural Products, AMR 2650-93, MRID No. 43358901.
5. Rühl, J. C., "Magnitude of Residues of Methomyl in Sorghum Forage and Hay Following Multiple Applications of Lannate® L Insecticide", DuPont Agricultural Products, AMR 2343-92, Supplement No. 1, in progress.

APPENDIX I
SUPPORTING RESIDUE SPREADSHEETS
(65% OF ORIGINAL SIZE)

DRY PEA SEED RECOVERY SPREADSHEET¹

SAMPLE ID NO.	SET NO.	FORT. LEVEL (ppm)	EXTRACT. DATE	HPLC DATE	SAMP WT. (g)	mL SOLV.	mL ALIQ.	FV * (mL)	PEAK HT.	m-VALUE**	b-VALUE†	HPLC DL FACT.	ng FOUND‡	METHOMYL (ppm)	CORRECTED METHOMYL§ (ppm)	f
S00105218 CK	1		07/15/94	07/18/94	10.00	100	5.00	1.00	0	1096	-28.1	1	0	0.00000	0.00	0
S00105218 SPK 1	1	0.020	07/15/94	07/18/94	10.00	100	5.00	1.00	451	1096	-28.1	1	0.437	0.01748	0.017	85
S00105218 SPK1 DUP	1	0.020	07/15/94	07/18/94	10.00	100	5.00	1.00	429	1096	-28.1	1	0.417	0.01668	0.017	85
S00105218 SPK 2	1	0.20	07/15/94	07/18/94	10.00	100	5.00	1.00	2093	1096	-28.1	2	1.936	0.15488	0.15	75
S00105218 SPK2 DUP	1	0.20	07/15/94	07/18/94	10.00	100	5.00	1.00	2107	1096	-28.1	2	1.948	0.15584	0.16	80
S00105218 SPK 3	1	2.00	07/15/94	07/18/94	10.00	100	5.00	1.00	2151	1096	-28.1	20	1.988	1.59040	1.6	80
S00105218 SPK3 DUP	1	2.00	07/15/94	07/18/94	10.00	100	5.00	1.00	2227	1096	-28.1	20	2.057	1.64560	1.6	80
S00105218 CK	4		07/26/94	07/27/94	10.00	100	5.00	1.00	0	990.5	30.42	1	0	0.00000	0.00	0
S00105218 SPK A	4	0.020	07/26/94	07/27/94	10.00	100	5.00	1.00	440	990.5	30.42	1	0.413	0.01652	0.017	85
S00105218 SPK B	4	0.20	07/26/94	07/27/94	10.00	100	5.00	1.00	4512	990.5	30.42	1	4.524	0.18096	0.18	90
S00105218 CK	4R		07/26/94	07/28/94	10.00	100	5.00	1.00	0	966.0	54.11	1	0	0.00000	0.00	0
S00105218 SPK B	4R	0.20	07/26/94	07/28/94	10.00	100	5.00	1.00	4507	966.0	54.11	1	4.609	0.18436	0.18	90

* FV (mL) = final volume (mL)

** m-value: slope of standard curve

† b-value: intercept of standard curve

‡ ng/mL found: concentration methomyl (ng/mL); concentration is calculated by the integrator before the peak height, m- and b-values are rounded

§ corrected methomyl (ppm): rounded to two significant figures

¹ DuPont Agricultural Products AMR 2566-93, "Magnitude of Residues of Methomyl in Pea Seed and Hay Following Application of Lannate® LV Insecticide", authored by D. M. Tomic.

DRY PEA HAY RECOVERY SPREADSHEET¹

SAMPLE ID NO.	SET NO.	FORT. LEVEL (ppm)	EXTRACT. DATE	HPLC DATE	SAMP WT. (g)	mL SOLV.	mL ALIQ.	FV * (mL)	PEAK HT.	m-VALUE**	b-VALUE†	HPLC DIL. FACT.	ng FOUND‡	METHOMYL (ppm)	CORRECTED METHOMYL§ (ppm)	% REC
S00105203 CK	2		07/18/94	07/21/94	10.00	100	5.00	1.00	43	932.5	10.41	1	0.00140	0.00140	0	0
S00105203 SPK 1	2	0.020	07/18/94	07/21/94	10.00	100	5.00	1.00	431	932.5	10.41	1	0.451	0.01804	0.018	90
S00105203 SPK1 DUP	2	0.020	07/18/94	07/21/94	10.00	100	5.00	1.00	423	932.5	10.41	1	0.442	0.01768	0.018	90
S00105203 SPK 2	2	0.50	07/18/94	07/21/94	10.00	100	5.00	1.00	1955	932.5	10.41	5	2.085	0.417	0.42	84
S00105203 SPK2 DUP	2	0.50	07/18/94	07/21/94	10.00	100	5.00	1.00	1901	932.5	10.41	5	2.028	0.40560	0.41	82
S00105203 SPK 3	2	2.00	07/18/94	07/21/94	10.00	100	5.00	1.00	2061	932.5	10.41	20	2.199	1.75920	1.8	90
S00105203 SPK3 DUP	2	2.00	07/18/94	07/21/94	10.00	100	5.00	1.00	1936	932.5	10.41	20	2.065	1.65200	1.7	85
S00105203 CK	3		07/25/94	07/26/94	10.00	100	5.00	1.00	0	879.2	1.799	1	0	0.00000	0.00	0
S00105203 SPK A	3	0.020	07/25/94	07/26/94	10.00	100	5.00	1.00	431	879.2	1.799	1	0.488	0.01952	0.020	100
S00105203 SPK B	3	0.50	07/25/94	07/26/94	10.00	100	5.00	1.00	1932	879.2	1.799	5	2.208	0.44160	0.44	88
S00105203 CK	3R		07/25/94	07/27/94	10.00	100	5.00	1.00	0	988.1	-17.6	1	0	0.00000	0.00	0
S00105203 SPK B	3R	0.50	07/25/94	07/27/94	10.00	100	5.00	1.00	2043	988.1	-17.6	5	2.085	0.41700	0.42	84

* FV (mL) = final volume (mL)

** m-value: slope of standard curve

† b-value: Intercept of standard curve

‡ ng/mL found; concentration methomyl (ng/mL); concentration is calculated by the integrator before the peak height, m- and b-values are rounded

§ corrected methomyl (ppm): rounded to two significant figures

¹ DuPont Agricultural Products AMR 2566-93, "Magnitude of Residues of Methomyl in Pea Seed and Hay Following Application of Lannate® LV Insecticide", authored by D. M. Tomic.

SORGHUM FORAGE RECOVERY SPREADSHEET

SAMPLE ID NO.	SET NO.	FORT. LEVEL (ppm)	EXTRACT. DATE	HPLC DATE	SAMP WT. (g)	mL SOLV.	mL ALIQ.	FV * (mL)	PEAK HT.	m-VALUE**	b-VALUE†	HPLC DIL. FACT.	ng/mL FOUND‡	METHOMYL (ppm)	CORRECTED METHOMYL§ (ppm)	% REC
S00035955 CK	1		04/12/94	04/15/94	10.00	100	5.00	1.00	64	41.81	8.336	1	1.331	0.00266	0.00	0
S00035955 SPK A	1	0.020	04/12/94	04/15/94	10.00	100	5.00	1.00	452	41.81	8.336	1	10.612	0.02122	0.021	105
S00035955 SPKA DUP	1	0.020	04/12/94	04/15/94	10.00	100	5.00	1.00	429	41.81	8.336	1	10.07	0.02014	0.020	100
S00035955 SPK B	1	0.10	04/12/94	04/15/94	10.00	100	5.00	1.00	2043	41.81	8.336	1	48.659	0.09732	0.097	97
S00035955 SPKB DUP	1	0.10	04/12/94	04/15/94	10.00	100	5.00	1.00	1749	41.81	8.336	1	41.643	0.08329	0.083	83
S00035955 SPK C	1	1.00	04/12/94	04/15/94	10.00	100	5.00	1.00	1925	41.81	8.336	10	45.853	0.91706	0.92	92
S00035955 SPKC DUP	1	1.00	04/12/94	04/15/94	10.00	100	5.00	1.00	1939	41.81	8.336	10	46.171	0.92342	0.92	92
S00035955 CK	3		05/06/94	05/10/94	10.00	100	5.00	1.00	0	35.85	21.30	1	0	0.00000	0.00	0
S00035955 SPK A	3	0.020	05/06/94	05/10/94	10.00	100	5.00	1.00	333	35.85	21.30	1	8.704	0.01741	0.017	85
S00035955 SPK B	3	0.10	05/06/94	05/10/94	10.00	100	5.00	1.00	1368	35.85	21.30	1	37.565	0.07513	0.075	75
S00035955 SPK C	3	1.00	05/06/94	05/10/94	10.00	100	5.00	1.00	1548	35.85	21.30	10	42.586	0.85172	0.85	85

* FV (mL) = final volume (mL)

** m-value: slope of standard curve

† b-value: intercept of standard curve

‡ ng/mL found: concentration methomyl (ng/mL); concentration is calculated by the integrator before the peak height, m- and b-values are rounded

§ corrected methomyl (ppm): rounded to two significant figures

SORGHUM HAY RECOVERY SPREADSHEET

SAMPLE ID NO.	SET NO.	FORT. LEVEL (ppm)	EXTRACT. DATE	HPLC DATE	SAMP WT. (g)	mL SOLV.	mL ALIQ.	FV * (mL)	PEAK HT.	m- VALUE**	b- VALUE†	HPLC DIL. FACT.	ng/mL FOUND‡	METHOMYL (ppm)	CORRECTED METHOMYL§ (ppm)	% REC
S00032951 CK	2		04/13/94	04/15/94	10.00	100	5.00	1.00	0	41.60	-7.75	1	0	0	0.00	0
S00032951 SPK A	2	0.020	04/13/94	04/15/94	10.00	100	5.00	1.00	405	41.60	-7.75	1	9.93	0.01886	0.020	100
S00032951 SPK A DUP	2	0.020	04/13/94	04/15/94	10.00	100	5.00	1.00	396	41.60	-7.75	1	9.706	0.019412	0.019	95
S00032951 SPK B	2	0.10	04/13/94	04/15/94	10.00	100	5.00	1.00	1568	41.60	-7.75	1	37.88	0.07576	0.076	76
S00032951 SPK B DUP	2	0.10	04/13/94	04/15/94	10.00	100	5.00	1.00	1896	41.60	-7.75	1	45.764	0.091528	0.092	92
S00032951 SPK C	2	1.00	04/13/94	04/15/94	10.00	100	5.00	1.00	1835	41.60	-7.75	10	44.29	0.8858	0.89	89
S00032951 SPK C DUP	2	1.00	04/13/94	04/15/94	10.00	100	5.00	1.00	1648	41.60	-7.75	10	39.803	0.79606	0.80	80
S00032951 CK	4		05/09/94	05/11/94	10.00	100	5.00	1.00	0	38.58	-14.2	1	0	0	0.00	0
S00032951 SPK A	4	0.020	05/09/94	05/11/94	10.00	100	5.00	1.00	347	38.58	-14.2	1	9.354	0.018708	0.018	95
S00032951 SPK B	4	0.10	05/09/94	05/11/94	10.00	100	5.00	1.00	1765	38.58	-14.2	1	46.125	0.09225	0.092	92
S00032951 SPK C	4	1.00	05/09/94	05/11/94	10.00	100	5.00	1.00	1733	38.58	-14.2	10	45.295	0.9059	0.91	91

* FV (mL) = final volume (mL)

** m-value: slope of standard curve

† b-value: intercept of standard curve

‡ ng/mL found: concentration methomyl (ng/mL); concentration is calculated by the integrator before the peak height, m- and b-values are rounded

§ corrected methomyl (ppm): rounded to two significant figures

SOYBEAN HAY RECOVERY SPREADSHEET¹

SAMPLE ID NO.	SET NO.	FORT. LEVEL (ppm)	EXTRACT. DATE	HPLC DATE	SAMP WT. (g)	mL SOLV.	mL ALQ.	FV * (mL)	PEAK HT.	m-VALUE**	b-VALUE†	HPLC DR. FACT.	ng FOUND‡	METHOMYL (ppm)	CORRECTED METHOMYL§ (ppm)	R
S00182016 CK	1		04/29/94	05/03/94	10.00	100	5.00	1.00	0	33.97	10.14	1	0	0.00000	0.00	0
S00182016 SPK A	1	0.02	04/28/94	05/03/94	10.00	100	5.00	1.00	316	33.97	10.14	1	9.004	0.01801	0.018	90
S00182016 SPK A DUP	1	0.02	04/28/94	05/03/94	10.00	100	5.00	1.00	305	33.97	10.14	1	8.69	0.01738	0.017	85
S00182016 SPK B	1	0.20	04/29/94	05/03/94	10.00	100	5.00	1.00	1631	33.97	10.14	2	47.704	0.19082	0.19	95
S00182016 SPK B DUP	1	0.20	04/29/94	05/03/94	10.00	100	5.00	1.00	1649	33.97	10.14	2	48.253	0.19301	0.19	95
S00182016 SPK C	1	1.00	04/29/94	05/03/94	10.00	100	5.00	1.00	1669	33.97	10.14	10	48.842	0.97684	0.98	98
S00182016 SPK C DUP	1	1.00	04/29/94	05/03/94	10.00	100	5.00	1.00	1579	33.97	10.14	10	46.173	0.92346	0.92	92
S00095117 CK	2		05/04/94	05/10/94	10.00	100	5.00	1.00	204	37.52	3.663	1	5.339	0.01068	0.011	0
S00095117 SPK A	2	0.02	05/04/94	05/10/94	10.00	100	5.00	1.00	553	37.52	3.663	1	14.649	0.02930	0.029	145
S00095117 SPK B	2	10.00	05/04/94	05/10/94	10.00	100	5.00	1.00	1768	37.52	3.663	100	47.022	9.40440	9.4	94
S00100032 CK	3		05/06/94	05/10/94	10.00	100	5.00	1.00	2085	59.64	28.81	1	34.481	0.06898	0.069	0
S00100032 SPK A	3	0.20	05/06/94	05/10/94	10.00	100	5.00	1.00	4016	59.64	28.81	2	66.851	0.26740	0.27	135
S00100032 SPK B	3	1.00	05/06/94	05/10/94	10.00	100	5.00	1.00	3221	59.64	28.81	10	53.527	1.07054	1.1	110
S00049975 CK	4		05/10/94	05/12/94	10.00	100	5.00	1.00	0	35.24	4.847	1	0	0.00000	0.00	0
S00049975 SPK A	4	0.02	05/10/94	05/12/94	10.00	100	5.00	1.00	348	35.24	4.847	1	9.737	0.01947	0.019	95
S00049975 SPK B	4	0.50	05/10/94	05/12/94	10.00	100	5.00	1.00	1739	35.24	4.847	5	49.197	0.49197	0.49	98
S00182016 CK	5		05/12/94	05/16/94	10.00	100	5.00	1.00	0	37.90	4.162	1	0	0.00000	0.00	0
S00182016 SPK A	5	0.02	05/12/94	05/16/94	10.00	100	5.00	1.00	371	37.90	4.162	1	9.87	0.01834	0.019	95
S00182016 SPK B	5	0.10	05/12/94	05/16/94	10.00	100	5.00	1.00	1753	37.90	4.162	1	46.15	0.09230	0.092	92
S00182024 CK	6		05/16/94	05/17/94	10.00	100	5.00	1.00	0	37.52	-11.6	1	0	0.00000	0.00	0
S00182024 SPK A	6	0.05	05/16/94	05/17/94	10.00	100	5.00	1.00	917	37.52	-11.6	1	24.759	0.04952	0.05	100
S00182024 SPK B	6	0.10	05/16/94	05/17/94	10.00	100	5.00	1.00	1731	37.52	-11.6	1	46.435	0.09287	0.093	93
S00182012 CK	7		05/18/94	05/19/94	10.00	100	5.00	1.00	0	45.83	-18.00	1	0	0.00000	0.00	0
S00182012 SPK A	7	0.02	05/18/94	05/19/94	10.00	100	5.00	1.00	424	45.83	-18.00	1	9.645	0.01929	0.019	95
S00182012 SPK B	7	0.10	05/18/94	05/19/94	10.00	100	5.00	1.00	1949	45.83	-18.00	1	42.926	0.08585	0.086	86
S00036643 CK	8		05/20/94	05/24/94	10.00	100	5.00	1.00	0	60.51	21.98	1	0	0.00000	0.00	0
S00036643 SPK A	8	0.02	05/20/94	05/24/94	10.00	100	5.00	1.00	584	60.51	21.98	1	9.288	0.01858	0.019	95
S00036643 SPK B	8	0.10	05/20/94	05/24/94	10.00	100	5.00	1.00	2760	60.51	21.98	1	45.247	0.09049	0.09	90
S00127182 CK	9		05/24/94	05/25/94	10.00	100	5.00	1.00	0	57.18	1.214	1	0	0.00000	0.00	0
S00127182 SPK A	9	0.02	05/24/94	05/25/94	10.00	100	5.00	1.00	529	57.18	1.214	1	9.236	0.01847	0.018	90
S00127182 SPK B	9	0.05	05/24/94	05/25/94	10.00	100	5.00	1.00	1397	57.18	1.214	1	24.417	0.04883	0.049	98

* FV (mL) = final volume (mL)

** m-value: slope of standard curve

† b-value: intercept of standard curve

‡ ng/mL found: concentration methomyl (ng/mL); concentration is calculated by the integrator before the peak height, m- and b-values are rounded

§ corrected methomyl (ppm): rounded to two significant figures

¹ DuPont Agricultural Products AMR 2650-93, "Magnitude of Residues of Methomyl in Soybean Hay Following Application of Lannate® Insecticide at Maximum Label Rates", authored by J. C. Rühl.

SUGAR BEET FOLIAGE RECOVERY SPREADSHEET¹

SAMPLE ID NO.	SET NO.	FORT. LEVEL (ppm)	EXTRACT. DATE	HPLC DATE	SAMP WT. (g)	mL SOLV.	mL ALQ.	FV * (mL)	PEAK HT.	m-VALUE**	b-VALUE†	HPLC DEL. FACT.	ng/mL FOUND‡	METHOMYL (ppm)	CORRECTED METHOMYL§ (ppm)	% REC
S00182008 CK	1		04/13/94	04/15/94	10.0	100	5.0	1.0	0	43.76	-22.3	1	0	0.00000	< LOQ	0
S00182008 SPK A	1	0.020	04/13/94	04/15/94	10.0	100	5.0	1.0	409	43.76	-22.3	1	9.863	0.01973	0.020	100
S00182008 SPK A DUP	1	0.020	04/13/94	04/15/94	10.0	100	5.0	1.0	411	43.76	-22.3	1	9.894	0.01979	0.020	100
S00182008 SPK B	1	0.20	04/13/94	04/15/94	10.0	100	5.0	1.0	1829	43.76	-22.3	2	42.315	0.16926	0.17	85
S00182008 SPK B DUP	1	0.20	04/13/94	04/15/94	10.0	100	5.0	1.0	1819	43.76	-22.3	2	42.071	0.16828	0.17	85
S00182008 SPK C	1	1.00	04/13/94	04/15/94	10.0	100	5.0	1.0	1813	43.76	-22.3	10	41.949	0.83898	0.84	84
S00182008 SPK C DUP	1	1.00	04/13/94	04/15/94	10.0	100	5.0	1.0	1465	43.76	-22.3	10	33.996	0.67892	0.68	88
S00062348 CK	2		04/18/94	04/20/94	10.0	100	5.0	1.0	0	45.51	8.345	1	0	0.00000	< LOQ	0
S00062348 SPK A	2	0.020	04/18/94	04/20/94	10.0	100	5.0	1.0	437	45.51	8.345	1	9.425	0.01885	0.019	95
S00062348 SPK B	2	1.00	04/18/94	04/20/94	10.0	100	5.0	1.0	2020	45.51	8.345	10	44.189	0.88398	0.88	88
S00062348 CK	2R		04/18/94	04/21/94	10.0	100	5.0	1.0	0	44.70	13.14	1	0	0.00000	< LOQ	0
S00062348 SPK B	2R	1.00	04/18/94	04/21/94	10.0	100	5.0	1.0	1981	44.70	13.14	10	44.034	0.88068	0.88	88
S00062356 CK	3		04/20/94	04/25/94	10.0	100	5.0	1.0	0	43.14	27.89	1	0	0.00000	< LOQ	0
S00062356 SPK A	3	0.020	04/20/94	04/25/94	10.0	100	5.0	1.0	403	43.14	27.89	1	8.688	0.01738	0.017	85
S00062356 SPK B	3	0.20	04/20/94	04/25/94	10.0	100	5.0	1.0	1904	43.14	27.89	2	43.493	0.17397	0.17	85
S00099038 CK	4		04/22/94	04/27/94	10.0	100	5.0	1.0	0	43.42	10.85	1	0	0.00000	< LOQ	0
S00099038 SPK A	4	0.020	04/22/94	04/27/94	10.0	100	5.0	1.0	399	43.42	10.85	1	8.933	0.01787	0.018	90
S00099038 SPK B	4	0.50	04/22/94	04/27/94	10.0	100	5.0	1.0	1880	43.42	10.85	5	43.052	0.43052	0.43	86
S00078131 CK	5		04/26/94	04/29/94	10.0	100	5.0	1.0	0	42.68	9.18	1	0	0.00000	< LOQ	0
S00078131 SPK A	5	0.10	04/26/94	04/29/94	10.0	100	5.0	1.0	1875	42.68	9.18	1	43.734	0.08747	0.087	87
S00078131 SPK B	5	1.00	04/26/94	04/29/94	10.0	100	5.0	1.0	1699	42.68	9.18	10	39.608	0.79216	0.79	79
S00182008 CK	6		04/28/94	05/03/94	10.0	100	5.0	1.0	0	34.00	16.19	1	0	0.00000	< LOQ	0
S00182008 SPK A	6	0.50	04/28/94	05/03/94	10.0	100	5.0	1.0	1915	34.00	16.19	4	55.838	0.44670	0.45	90
S00182008 SPK B	6	1.00	04/28/94	05/03/94	10.0	100	5.0	1.0	1571	34.00	16.19	10	45.721	0.91442	0.91	91
S00078131 CK	7RA		05/04/94	05/09/94	10.0	100	5.0	1.0	0	36.12	30.41	1	0	0.00000	< LOQ	0
S00078131 SPK A	7RA	1.00	05/04/94	05/09/94	10.0	100	5.0	1.0	1500	36.12	30.41	10	40.688	0.81376	0.81	81
S00078131 SPK B	7RA	2.00	05/04/94	05/09/94	10.0	100	5.0	1.0	1528	36.12	30.41	20	41.483	1.65852	1.7	85
S00182008 CK	8RA		05/06/94	05/10/94	10.0	100	5.0	1.0	0	60.69	24.00	1	0	0.00000	< LOQ	0
S00182008 SPK A	8RA	1.00	05/06/94	05/10/94	10.0	100	5.0	1.0	2685	60.69	24.00	10	43.855	0.87710	0.88	88
S00182008 SPK B	8RA	3.00	05/06/94	05/10/94	10.0	100	5.0	1.0	4488	60.69	24.00	20	73.56	2.94240	2.9	97

* FV (mL) = final volume (mL)

** m-value: slope of standard curve

† b-value: intercept of standard curve

‡ ng/mL found: concentration methomyl (ng/mL); concentration is calculated by the integrator before the peak height, m- and b-values are rounded

§ corrected methomyl (ppm): rounded to two significant figures

¹ DuPont Agricultural Products AMR 2653-93, "Magnitude of Residues of Methomyl in Sugarbeet Foliage Following Application of Lannate® LV Insecticide", authored by J. C. Rühl.

APPENDIX II

EXTRACTION EFFICIENCY RAW DATA AND CALCULATIONS

Sample ID	Raw Counts (dpm)	Average Counts (dpm)	Total Volume (mL)	Total Counts (dpm)	% Recovery
Pea Hay-1	18634 18241 18238	18371	136	2495736	98.8
Pea Hay-2	17164 17231 16973	17123	150	2565450	102
Pea Seed-1	16507 16979 16952	16813	162	2720466	108
Pea Seed-2	17039 17126 16995	17053	158	2691214	93.8
Sorghum Fodder-1	17545 17577 17820	17647	148	2608796	103
Sorghum Fodder-2	17503 17735 17791	17676	146	2577776	102
Sorghum Hay-1	16559 16530 16654	16581	154	2550394	101
Sorghum Hay-2	17638 17828 17927	17798	142	2524476	100
Sugar Beet Foliage-1	17700 17326 17240	17422	150	2610300	103
Sugar Beet Foliage-2	16775 16592 16868	16745	158	2642550	105

Total Counts (dpm) was calculated by calculating the Average Counts (dpm) from the three 1.0-mL aliquots, subtracting the Background Counts (20 dpm), and multiplying by the total volume (mL):

$$\begin{aligned} \text{Total Counts (dpm)} &= [(\text{Average Counts} - \text{Background counts}), \text{dpm}] \times \text{Total Volume (mL)} \\ &= [(\text{Average Counts} - 20), \text{dpm}] \times \text{Total volume (mL)} \end{aligned}$$

Percent Extraction Efficiency was calculated by dividing the Total Counts in the supernatant solution by the Total Counts in the Fortification (2525700 dpm) and multiplying by 100 %:

$$\text{Percent Extraction Efficiency} = \frac{\text{Total counts (dpm)}}{\text{Fortification Counts (dpm)}} \times 100\% = \frac{\text{Total counts (dpm)}}{2525700 \text{ dpm}} \times 100\%$$

APPENDIX III
CONFIRMATORY METHOD INSTRUMENTATION, OPERATING
CONDITIONS AND SAMPLE CHROMATOGRAM

SENT BY:

9-14-94 ; 15:08 ; SCIEX THORNHILL CAN-

302 030 42301# 4/ 0



TABLE II - Instrument Operating Parameters

Instrumentation:

Waters 600-MS HPLC gradient pump
 Waters WISP 717 autosampler
 Finnigan MAT TSQ-700 equipped with Finnigan TSP2

HPLC Operating Conditions

Column: Waters Nova-Pak C₁₈, 3.9 x 150 mm column
 Injection Vol. 10 μ L
 Flow Rate: 1.2 ml/min

Time	Gradient	0.2M Ammonium Acetate	Methanol
Initial	-	90	10
10	Linear	40	60
10.1	Step	90	10

Mass Spectrometer Operating Parameters:

Vaporizer Temperature: 92°C
 Source Temperature: 250°C
 Collision Energy: -14 eV
 Collision Pressure: Argon @ approx. 1.7 mtorr

Analyte	MW	Ion(s) Monitored precursor - product (dwell)	Retention Time (approx. min.)
Methomyl	162	m/z 163.1 - m/z 87.9 (0.5 sec.) m/z 163.1 - m/z 105.9 (0.5 sec.)	5:47

SENT BY:

3-14-94 13:00 JCTA INSTITUTE

SEE SEE REPORT

QUAN: 940908-15.q0

Entries: 1

Data file(s) used

08-SEP-94

File: 940908-15

Start : 15:09:03 192

Samp: 13855-2

Comm: Set#1-S00107398 (0.5g/1.0ml) DFAC-1 TSQ-2

Study : Methomyl

Mode: TSP +DAU LMR GAS UP LR

Inlet : Vial 6

Oper: JDL

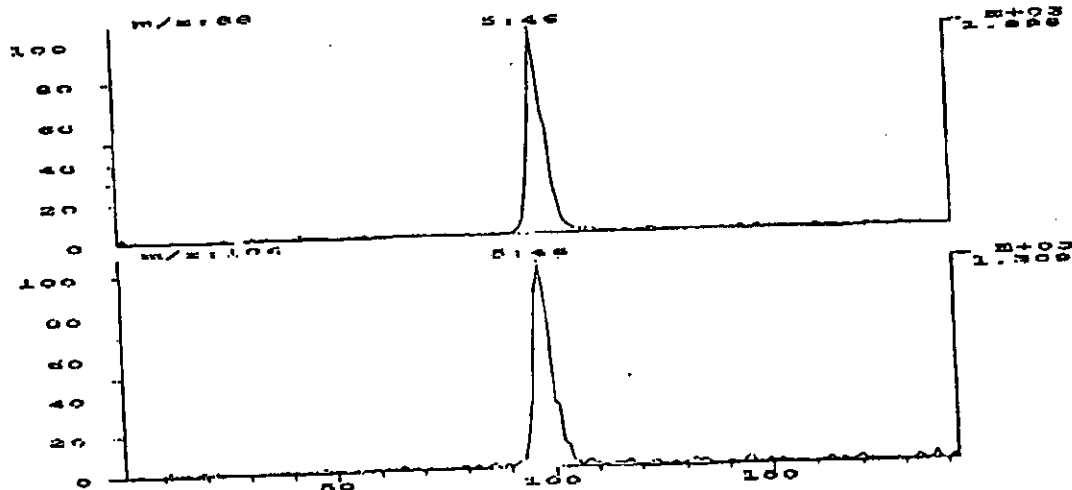
Client: Spk1 @ 0.020 ppm

Disp: SEAR CHRO

Amount = (area * ref amnt) / (ref area * response factor)

Response factors from average of response file (Last 3 pt) (1REF)

#	Compound Name	RT	DT	Area	Avg. RF	RSD	Quan Type	ATL	ANS	BW	MPR
1	Methomyl	5:46	2.4	15127.	1540.	5.8	ABB	4	2.	10	3



Methomyl
RIC

